



TLR2/1 Orchestrate Human Plasmacytoid Dendritic Cells Response to Gram+ Bacteria

Salvatore Raieli

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Salvatore Raieli. TLR2/1 Orchestrate Human Plasmacytoid Dendritic Cells Response to Gram+ Bacteria. Innate immunity. Université Paris Saclay (COMUE), 2016. English. NNT : 2016SACLS495 . tel-01656730

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NNT : 2016SACLS495

**THESE DE DOCTORAT
DE
L'UNIVERSITE PARIS-SACLAY
PREPAREE A
L'UNIVERSITE PARIS-SUD**

**ECOLE DOCTORALE N° 582
CBMS Cancérologie : biologie - médecine - santé**

Spécialité de doctorat : Aspects moléculaires et cellulaires de la biologie

**TLR2/1 orchestrate human plasmacytoid dendritic cells
response to Gram+ bacteria**

Par

Salvatore Raieli

Thèse présentée et soutenue à Paris, le 5 Décembre 2016

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Per aspera sic itur ad astra

Marcus Tullius Cicero

Acknowledgement

*Who built Thebes of the 7 gates ?
In the books you will read the names of kings.
Did the kings haul up the lumps of rock ?
And Babylon, many times demolished,
Who raised it up so many times ? [...]*

*The young Alexander conquered India.
Was he alone ?
Caesar defeated the Gauls.
Did he not even have a cook with him ?
Philip of Spain wept when his armada went
down.
Was he the only one to weep ?
Frederick the 2nd won the 7 Years War. [...]*

*Questions From a Worker Who Reads.
Bertolt Brecht (1935)*

Bertolt Brecht wrote this poem explaining that behind each enterprise there is more than one man, that every challenge requires a great team.

I want to thank Vassili for giving me the opportunity to develop a scientific, critical mind and for the great freedom to explore and test different challenges.

I want to thank the jury for their kind availability at this fundamental time in my personal growth. I want to thank the rapporteurs for their valuable comments and the critical reading of the manuscript allowing me to improve it.

I would like to thank the U932 unit for creating such a pleasant frame where to work.

I want to thank the Institut Curie Cytometry platform members: Zofia, Sophie and Annick for their time and their great help.

I would like to thank Vassili's "team family" who have supported me during the last few years : Lucia for her great suggestions, Max for his suggestions and his jokes, Paula, Solana, Coline, Philemon, Floriane, Maude, Philemon, Ares, Antonio, without forgetting past members: Alix, Mahe, Irit, Marine, Gerome, Eve. When will we have the next "aperol" in the lab?

I want to thank my coffee team (PhD metabolism is mostly based on coffee consumption): Matteo (I have to be short... I cannot write ten pages about why I want to thank you), Nicolas (my great Spanish teacher who gave me great suggestions also in science), François (who corrected my awful French), Francesca (after-lunch meeting mandatory) and another Nicolas (you are not coming to sort the cells... just for the coffee). Guys, how are you standing my peculiar sense of humor?

I want to thank my colleagues who made an effort to put up with me after lab time: Pamela, Leticia, Andres, Rodrigo, Emanuel, Antonella (south American communities whom I hope will accept me as a citizen), Michaelis, Vasco, Ester (I know, I am annoying),

I want to thank my English reviewer, I know it was a hard battle to found my errors (I have to be short I have much more to thank you): Lidia (my best Portuguese friend), Matthew (my great pool companion), Siba (my God, you should have killed me!), Anvita (crazy, annoying and super nice person) and Emilia Maggio (for her incredible corrections).

I want to thank my French class companion: Angelo (who has a terrible sense of humor, just like me... or even worse), Federica and Pablo (I have to thank you for many other things...I have not forgotten).

I want to thank the friends with whom I grew up together and their respective mates: Angelo (one of the best person I've ever met, could you imagine back then that we would do a PhD?) and Rosemary and Pino (brother, without your dinners Paris would have been an sadder place) and Azzurra and Marta.

I want to thank Francesco, who, like a swallow, migrates seasonally back and forth from Rome to Paris.

Thanks to my parents, Maria and my Vincenzo, my sister Chiara, my grandmothers Marisa and Candida, my uncles (Teresa, Beppe, Anna, Giuseppina and Massimo) and my cousins (Marialuisa, Pietro, Daniele, Francesco, Andrea, Natalia): I should write another chapter to be enough extensive to thanks you. I want to thank my aunt Anna – you should have been here but I know that in one way you are with us.

I want to thank my girlfriend Gigliola, who has supported me during my PhD and without you it wouldn't be the same story, how we did not kill each other writing our theses at the same time?

Thanks again, all of you for these 4 faboulus years.

Salvo

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Preamble

Two things fill the mind with ever-increasing wonder and awe, the more often and the more intensely the mind of thought is drawn to them: the starry heavens above me and the moral law within me.

Immanuel Kant, Critique of Practical Reason, 1788

When Immanuel Kant wrote this inspiring quote, fascinated by the complexity of the starry heavens, biology was moving its first steps. Nowadays, we can appreciate the intricate complexity of the living system, crafted by billions of years of evolution. In this outstanding landscape I was fascinated by the immune system and its elaborate strategies designed for confronting daily threats. This system relies on two components: innate immunity as a first line of defense, common to all the vertebrates, to quickly counterattack the invading microbes, and adaptive immunity as a sophisticated system that evolves to confront new threats. This system bases its efficiency on its complicate regulation and its capacity to be flexible to adapt to a wide range of challenges.

In the 20th century the positivistic idea of science was undermined by the formulation of Heisenberg's Uncertainty principle and by quantum mechanics in general. In the 30's, Gödel's Incompleteness theorems revolutionized math to the extent of affecting our faith in it. At the same time, the concept that the immune system can be detrimental to its host when its delicate balance is altered can also be considered revolutionary. As physicists have abandoned the simplicity of the Newton mechanics to delve into the uncertain space of quantum theories, more in-depth studies and a new way of thinking appear to be necessary in order to understand how all the parts of the immune system can work together in concert.

As I was captivated by this compelling challenge, my thesis is focused on the pivotal role of dendritic cells in determining the final outcome of the immune response. These cells are in a key position between innate and adaptive immunity: their repertoire of sensors allows them to sense potential threats in order to mount a specific response or shoot down excessive responses to harmless antigens. This study is mainly based on a particular subset, the plasmacytoid dendritic

cells (pDCs), and their interaction with GRAM+ bacteria. The unique features of pDCs and their capacities of interaction with all the immune system components influence their responses. The aim of my work was to decipher how they sense GRAM+ bacteria, which sensors are implied, and how they affect pDCs response.

This manuscript is divided in three parts: an Introduction, describing the state of the arts about pDCs, their response to bacteria and TLR2/1, to outline the framework in which results will be inserted. The results, in the form of a manuscript finalized for submission, describe how pDCs sense GRAM+ bacteria, as well as the impact of TLR2/1 on pDC function. In the discussion and perspectives results will be discussed within the wider frame of their implications and participation, with the aim of answering some open questions.

List of abbreviations

ALRs	AIM2-like receptors
AMP	adenosine monophosphate
AMPs	anti-microbial peptides
APC	antigen presenting cells
Aβ	Amyloid β
BALF	bronchoalveolar lavage fluid
BBB	blood-brain barrier
BCG	Bacillus Calmette-Guérin
BDCA	blood dendritic cells antigen
BM	bone marrow
CCL2	CC-chemokine ligand 2
CCR	chemokine receptor
CD	Crohn's disease
CDP	common dendritic cell precursor
CLP	common lymphoid precursors
CMP	common myeloid precursors
CNS	central nervous system
CSF	cerebrospinal fluid
CTLA-4	cytotoxic T lymphocyte-associated protein 4
CXCL1	CXC-chemokine ligand 1
CXCL2	CXC-chemokine ligand 2
DAP6	domain-associated protein 6
DAMPs	danger-associated molecular patterns
DCs	dendritic cells
DM	Dermatomyositis
dsDNA	double-stranded DNA
dsRNA	double-stranded RNA
DT	diphtheria toxin
DTR	diphtheria toxin receptor
EAE	Experimental autoimmune encephalomyelitis
FLT3L	fms-like tyrosine kinase-3 (Flt3) ligand
FRET	fluorescence resonance energy transfer
GC	germinal center
GITRL	glucocorticoid-induced tumor necrosis factor receptor-ligand
HEV	high endothelial venules
Hla	α -hemolysin
HSC	hematopoietic stem cells
hsp60	heat shock protein 60

HSV-1	herpes simplex virus type 1
IBD	inflammatory bowel disease
ICD	intracellular domain
ICOSL	inducible T-cell co-stimulator ligand
IDO	indoleamine 2,3-dioxygenase
IFN	interferon
IHC	immunohistochemistry
IKK	I κ B kinase
IL	interleukine
ILT7	immunoglobulin-like transcript 7
IPCs	Type 1 interferon-(α , β , ω)-producing cells (IPCs)
LAIR1	leukocyte-associated immunoglobulin-like receptor 1
LAT	lipoteichoic acids
LBP	LPS-binding protein
LCs	Langerhans cells
LDL	low density lipoproteins
LLT1	lectin-like transcript 1
LN	Lymph node
LPP	lipoprotein
LPS	lipopolysaccharide
LRR	leucine-rich repeat
LTA	lipoteichoic acid
MAB	<i>Mycobacterium abscessus</i>
MAP3K8	MAPK kinase kinase 8
MDA5	melanoma differentiation antigen 5
mDCs	myeloid DCs
MG	Myasthenia gravis
MHC	major histocompatibility complex
MIP	<i>Mycobacterium indicus pranii</i>
MMP	Matrix metalloprotease
MPO	Myeloperoxidase
MS	Multiple sclerosis
MTB	<i>Mycobacterium Tuberculosis</i>
MVs	called membrane vesicles
NETs	neutrophils extracellular traps
NKT	Natural Killer T
NMJ	neuromuscular junction
NO	nitric oxide
PAMPs	pathogen-associated molecular patterns
PAUF	Pancreatic adenocarcinoma up-regulated factor

PD-1	programmed cell death protein 1
pDCs	plasmacytoid dendritic cells
PD-L1	programed death receptor-ligand 1
pETs	pDCs extracellular traps
PGE2	Prostaglandin 2
PGN	peptidoglycan
PI3K	Phosphatidylinositide 3-kinase
PIP3	phosphatidylinositol-(3,4,5)-trisphosphate
poly(I:C)	polyinosinic–polycytidylic acid
PPD	purified protein derivate
PPE	proline-proline-glutamic
PRRs	Pathogen-recognition receptors
PSA	polysaccharide A
RIG-I	retinoic acid-inducible gene-I
RLH	RIG-like helicases
ROS	reactive oxygen species
SAA	Serum amyloid A
SARM	Sterile α and Armadillo motif
SLE	Systemic lupus erythematosus
SNP	single nucleotide polymorphism
SpA	Staphylococcal protein A
SS	Sjögren's syndrome
SSc	Systemic sclerosis
ssRNA	single-stranded form of RNA
T1D	Type I diabetes
TAK1	transforming growth factor- β (TGF- β)-activated kinase 1
TB	tuberculosis
TB	tuberculosis
TCR	T cell receptor
TDM	trehalose-6,6' dimycolate
Tfh	T follicular helper
TGF-β	transforming growth factor β
TH	T helper
TIR	Toll–interleukin 1 receptor
TLR	Toll like receptors
TPO	Thrombopoietin
TRAIL	tumor necrosis factor-related apoptosis-inducing ligand
TSTs	Tuberculin skin tests
UC	ulcerative colitis
β2-gpl	β 2-Glycoprotein I

INTRODUCTION

1. Plasmacytoid dendritic cells and their role in immunity

1.1 Dendritic cells: A glance to the past

In 1868 Paul Langerhans observed a new cell type in epidermal tissue sections that later was named the Langerhans cell (LC). Although he considered LCs a new type of neuron cells, the link between them and the immune system was highlighted many decades later. The history of the dendritic cell (DC) began when Ralph Steinman and Zanvil Cohn discovered a new cell type in the spleens of mice in 1973. They named it dendritic cell (DC) due to its cytoplasmic extensions that reminded them of a tree. The enrichment of this population prompted them to conduct functional studies that revealed the cells' capacity to induce potent proliferation and activation in T-cells. In 1985 LCs were recognized to be part of the DCs family by Gerold Schuler and Ralph Steinman.

In 1991 Ralph Steinman proposed the criteria for defining a DC: 1) a dendritic morphology, (2) the constitutive expression of high levels of major histocompatibility complex (MHC) class two molecules, and (3) a capacity to induce proliferation of naïve CD4⁺ T cells in a mixed leukocyte reaction.

Since the discovery of DCs the scientific community's interest has grown exponentially, and an abundance of observations and studies has shown the importance of DCs in the immune system. This importance was definitely stated in 2011 with the Nobel Prize assigned to Steinman *"for his discovery of the dendritic cell and its role in adaptive immunity"*.

1.2 Dendritic cell characteristics and functions

In humans, unlike mice, where spleen and lymph nodes are available, the most accessible tissues for the study of leukocytes are peripheral blood and the skin. In 1982, after several studies, a rare population was identified in the human blood (accounting for less than 1% of the total PBMCs) that shared cytological characteristics, high levels of MHC-II and the capability of inducing potent activation of T-cells^[1]. DCs are defined as lineage negative (CD3- (T-cell), CD19/20 (B-cell), and

CD16 (natural killer (NK) cell)) and they are MHC-II high (the high level of expression of HLA-DR is used to exclude monocytes). [²]

DCs are antigen-presenting cells with the ability to shape the adaptive immune response according to the menaces they encounter. The circulating pool of human DCs is renewed on a regular basis from the bone marrow hematopoietic stem cells (HSCs). According to the current view, CD34+ HSCs are multi-potent progenitors that give rise to common lymphoid precursors (CLPs) and common myeloid precursors (CMPs); both are capable of generating monocytes, macrophages and DCs. The progenitor cells that eventually differentiate into DCs in human (despite a better knowledge in mice) are yet to be identified [^{3,4}]. The precursors enter the blood, acquire a mature phenotype and home to different tissues. The DCs reside in the tissues in an immature state, ready to sense the micro-environment thanks to their capacity to phagocytate and to the an abundance of sensors which they possess (that will be discussed in details below).

The current nomenclature divides the peripheral blood circulating DCs into three different subsets according to the expression of different surface antigens (BDCA, blood dendritic cells antigen): two myeloid DC subsets by the expression of BDCA-1(CD1c) and BDCA-3(CD141) and pDCs characterized by BDCA-2(CD303) and BDCA-4(CD304) [^{1, 5}].

DCs are capable of capturing antigens, expressing co-stimulatory molecules on the surface; they possess high levels of MHC-II complex, migrate to secondary lymphoid organs and secrete a high quantity of cytokines. Moreover, they can induce sustained CD4+ T-cell proliferation and shape their polarization based on the different T-helper subsets. DCs are also capable of presenting exogenous antigens to the CD8+ T-cells in a process called cross-presentation. In a different context, DCs are also capable of dampening the immune response or inducing tolerance towards certain antigens. In other words, DCs are in a unique position and they play an important role in the outcome of the whole immune response. [^{6,7}]

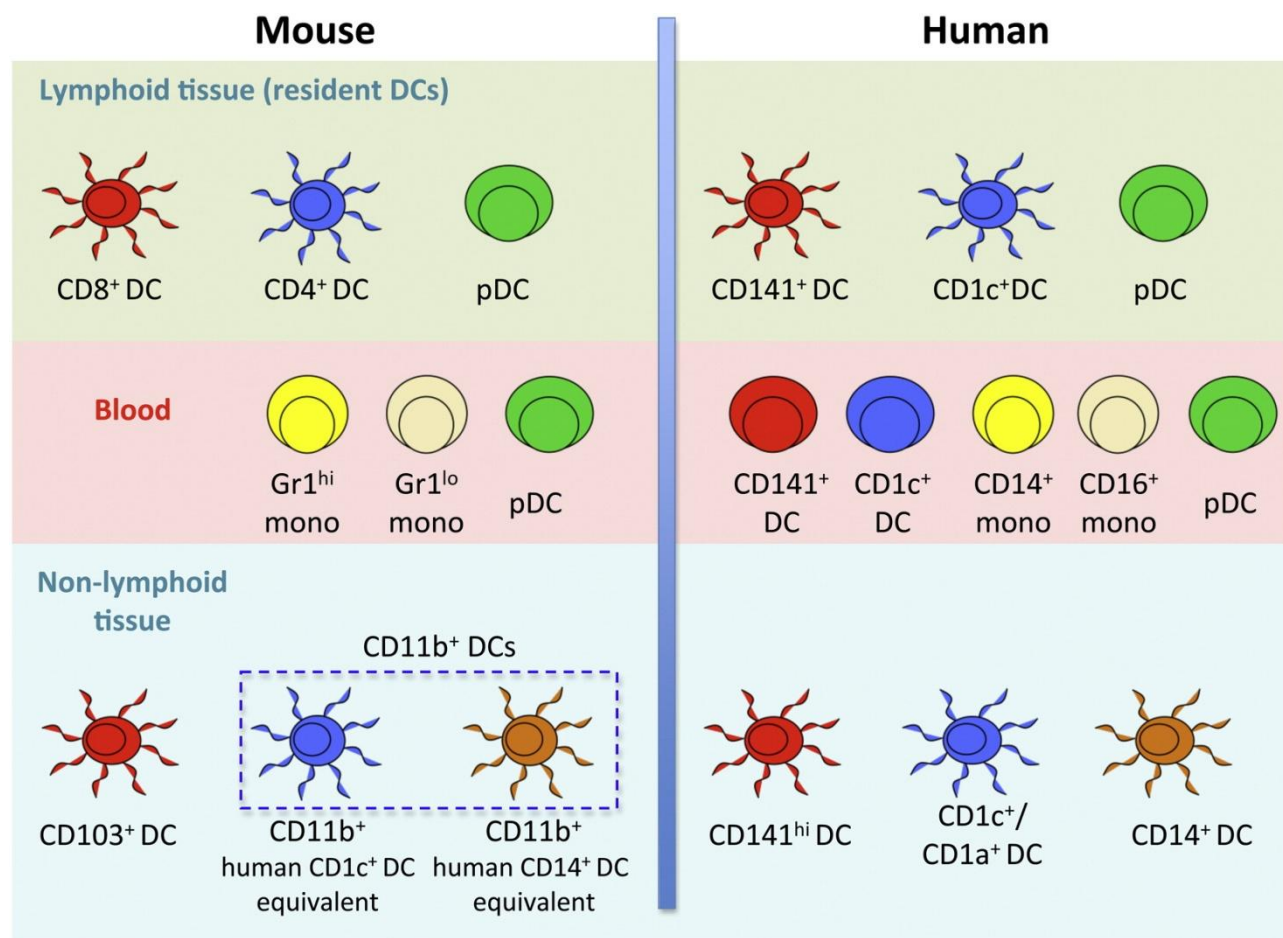


Figure 1: Dendritic cells subsets in mice and humans. From Haniffa et al., 2012

1.3 The discovery of plasmacytoid dendritic cells: an immunology spy story

In 1958 K. Lennert and W. Remmele observed a cell, within the T-cell area of lymph nodes, which they called “T-associated plasma cells” because of its plasma cell morphology. In 1983 it was found that these cells were expressing CD4 and not B cell antigen, leading the scientists to rename them “plasmacytoid T cells”. Further observations demonstrated that these cells were not expressing T cell receptor (TCR), and the scientific community renamed them “plasmacytoid monocytes” because of their expression of MHC class II molecules. In 1996/1997, in the T-cell zone present within tonsils around the high endothelial venules (HEV), CD4⁺CD3[−]CD11c[−] cells with lymphoid-

plasmacytoid morphology were found. These cells were able to differentiate into mature DCs and were later renamed “plasmacytoid dendritic cell precursors (pDCs)” [7].

In 1970, the presence of a blood cell type capable of secreting a higher amount of type I interferon (IFN) compared with other cell types was detected. Due to this peculiar characteristic, these cells were called Type 1 interferon-(α , β , ω)-producing cells (IPCs). To start with, scientists believed NK cells were exerting this secretion. Subsequent studies excluded NK cells and B cells, T cells, monocytes and macrophages from responsibility for IFN production. These IPCs were found to express high levels of MHC-II; they were thought to belong to the DC family. In 1999 the connection between IPCs and pDCs was finally made and it was accepted that indeed they were the same cells. [8]

1.4 Plasmacytoid dendritic cells characteristics

pDCs morphology

Under a light microscope, and through Giemsa staining, it is possible to observe that pDCs have a smooth, round morphology and that they resemble plasma cells. pDCs have a diameter between 8-10 μm , are smaller than monocytes and bigger than non-activated lymphocytes. Unlike monocytes, whose nucleus is horseshoe-shaped, pDCs have a kidney-shaped nucleus. They also have a basophilic cytoplasm and a dim Golgi zone. In transmission electronic microscope pictures it is possible to observe the nucleus with marginal heterochromatin, a well-developed rough endoplasmic reticulum, a reduced Golgi apparatus and the presence of many mitochondria. [9]

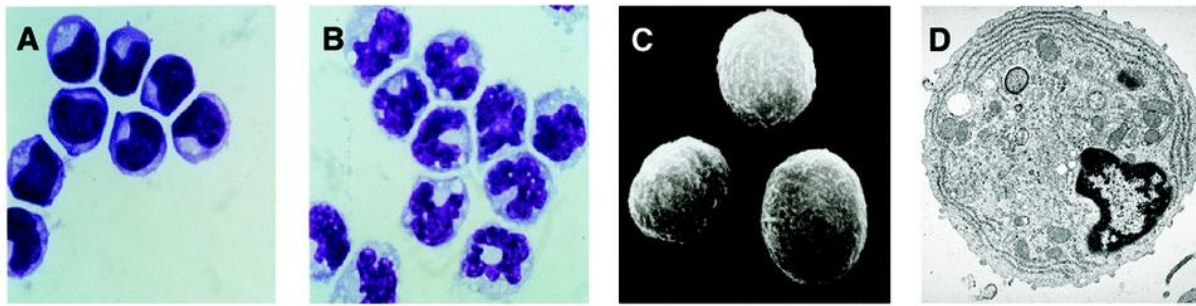


Figure 2: **A.** pDCs Giemsa staining. **B.** Monocytes Giemsa staining. **C.** Scanning electron microscopy picture of pDCs. **D.** Transmission electron microscopy picture of pDCs. Adapted from Liu et al., 2005.

pDCs development

Despite the wide interest in understanding the development of pDCs, there are still many open questions. The administration of fms-like tyrosine kinase-3 ligand (FLT3L) into healthy volunteers appears to promote pDCs development: after the injection there was a significant increase of peripheral blood pDCs. Likewise, FLT3L transgenic mice have a higher number of pDCs compared to the wild type. FLT3L knock-out mice have fewer pDCs. In addition, FLT3L may promote the survival of pDCs in the periphery and this effect is also shown in *in vitro* experiments. Mainly, FLT3L is believed to promote the generation of pDCs in the bone marrow. Cultures of mouse bone marrow-derived cells give rise to a good yield of pDCs after 5-9 days when treated with FLT3L. ^[10]

Other studies suggest that M-CSF can play a role in pDCs development: cultures of mouse bone marrow-derived cells can differentiate in pDCs when treated with M-CSF. On the contrary, other myeloid cytokines, such as GM-CSF, inhibit pDCs development. However, thrombopoietin (TPO), better known for its role in megakaryocyte/platelet development, promotes pDC development from precursors. It seems that TPO is acting in combination with FLT3L. IL-7 is also capable of promoting pDC development; their number of pDCs is reduced in IL-7 knock-out mice. It is thought that IL-7/IL-7R signaling plays a role during pDC development in the bone marrow. ^[11, 12, 13, 14]

The FLT3L signal passes through STAT3, a factor in the development of all DCs. IRF8 has proved crucial to the development of pDCs since IRF8 knock-out mice show depletion of these cells. E2-2 is specifically required for their development in both humans and mice. This transcription factor, a member of basic helix-loop-helix family, activates IRF8 and SpiB (another important gene involved in pDCs development). In the absence of E2-2 pDCs are not found in peripheral blood. [¹⁵, ¹⁶, ¹⁷, ¹⁸, ¹⁹]

pDCs can be efficiently generated by CMP and CLP precursors in the bone marrow: most CLPs are FLT3+; on the contrary, only a small portion of CMPs is FLT3+. Other evidence showed that pDCs can have lymphoid-related developmental history, such as the rearrangement of immunoglobulin heavy chain (IgH) genes (this phenomenon can be appreciated in the other lymphoid cells outside B-cells). This is not happening in the myeloid dendritic cells. [²⁰]

On the other hand, a common dendritic cell precursor (CDP) was found downstream of CMP, which suggested a myeloid origin for pDCs development. CDPs give origin to both pDCs and myeloid DCs. [²¹, ²²]

In conclusion, some differences between CLP- and CDP-derived pDCs were found. CDP pDCs in the mouse present different markers of mature pDCs such as CD11c, B220 and Siglec-H but they express low levels of MHC-II. CDP pDC progenitors do not express MCSF-R and maintain high levels of E2-2. CLP-derived pDCs show differences in IL-6, TNF- α and Type I IFN production compared to their CDP-derived counterpart. The question of whether pDCs have a myeloid or lymphoid origin is still open. [²³]

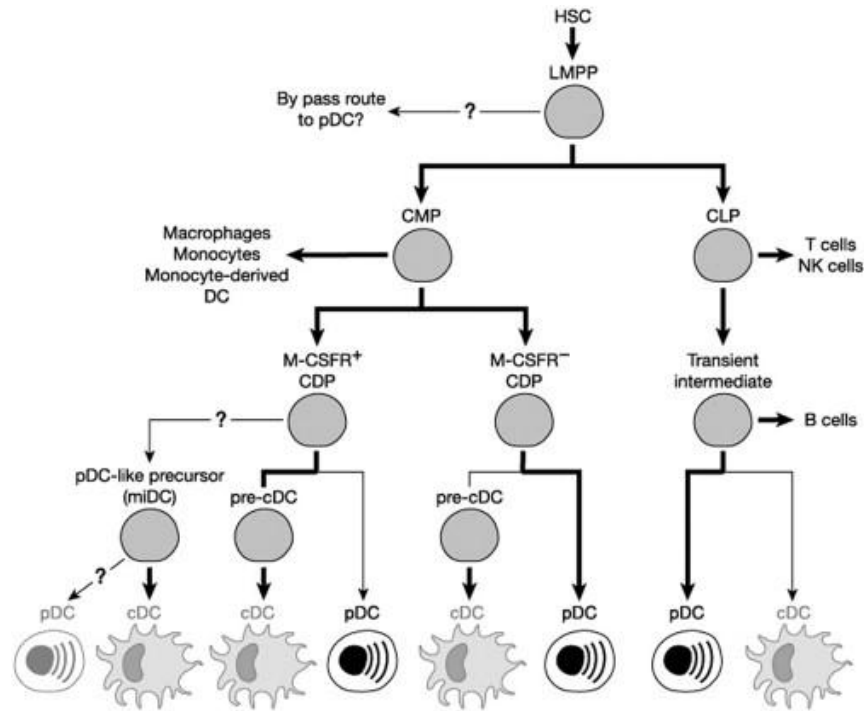


Figure 3: Possible developmental pathways to plasmacytoid cells. From Shortman et al., 2012

Localization, migration and life span of pDCs

pDCs are constantly produced in the bone marrow; pDCs circulate after their exit in the peripheral blood. pDCs migrate to the T-cell area in the lymph nodes through the high endothelial venules (HEV). On the contrary, the other DCs migrate through the afferent lymphatic vessels to the lymph nodes. pDCs also migrate in a homeostatic state to mucosa-associated lymphoid tissues and the marginal zone of the spleen. pDCs can also migrate to peripheral tissues. [24, 25]

pDCs constitutively express CD62L (L-selectin for lymph node homing) and PSGL1. Moreover, their unique ability to migrate through HEV seems related to the fact that they express CXCR4 (a chemokine receptor with CXCL12 as a ligand; this chemokine is produced by HEV but it can also be expressed by the skin). pDCs also express CCR9 (the chemokine receptor for CCL25), which plays a role in the homing to the small intestine. When pDCs are activated, they express other receptors that influence their migration. They up-regulate the expression of CCR7 for lymph node homing, CCR5, CXCR3 and $\beta 1/\beta 2$ integrins, receptors for CCL19 and CCL21 (a chemokine produced by the

HEV). pDCs also express CXCR3 and CCR9, two receptors respectively guiding the pDCs towards the skin and the small intestine. pDCs can also migrate to inflamed tissues: IL-3 stimulation leads them to express CCR6 and CCR10 that drive them towards inflamed skin or mucosa. ^[26, 27] Actually, pDCs migration is influenced by the environment, and different activation states lead to a different recruitment.

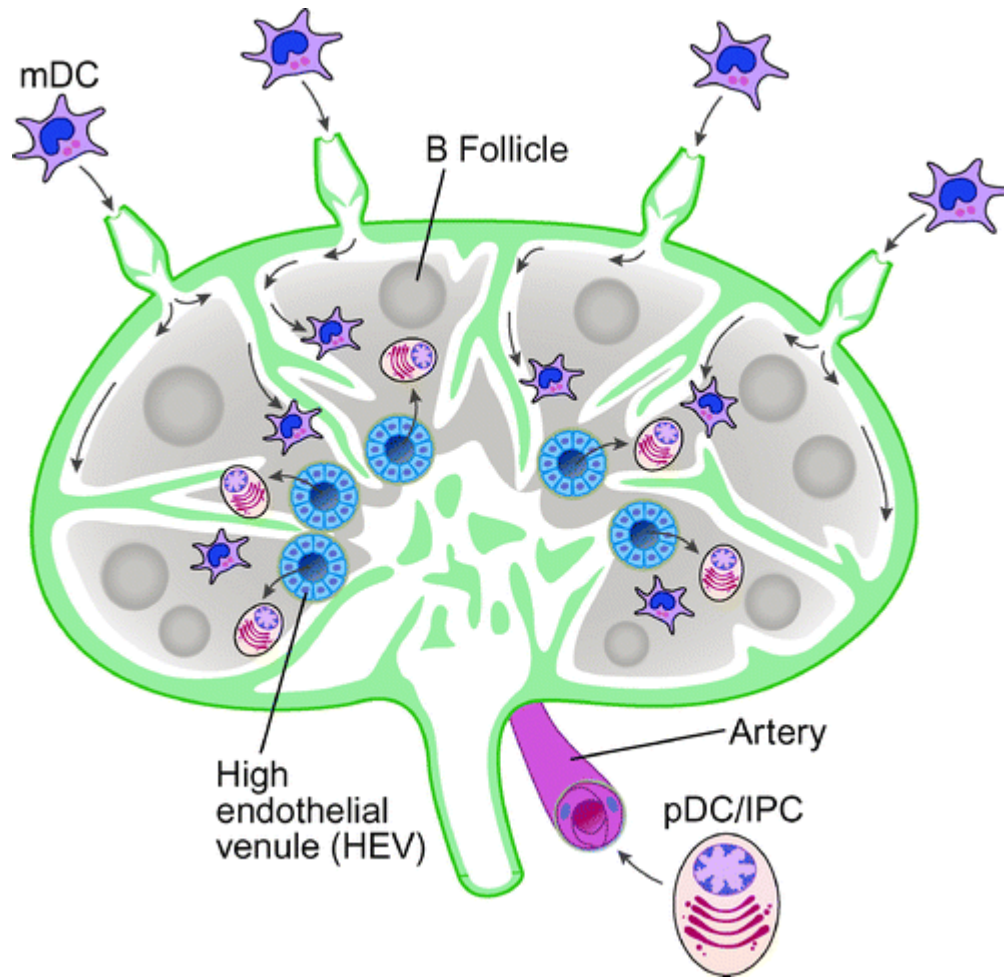


Figure 4: Migration of pDCs in the lymph nodes. From Liu et al., 2005

1.5 pDCs, a node in an intricate network

Although pDCs are mainly known for the high amount of Type I IFN they can secrete, it is reductive to consider their role in immunity as limited to this. In this chapter, it would be interesting to

summarize their capability to interact with a plethora of different cells through cytokine production and/or direct interaction. All these elements allow us to conclude that pDC activation can influence the different branches of the immune system: from innate to adaptive immunity.

Endothelial cells

After exiting the bone marrow, pDCs enter the blood stream. Endothelial cells have been shown to express different cell surface molecules and to secrete chemokines and cytokines that may aid pDCs and other leukocytes transmigration and regulate their activation state. pDCs are facilitated in their migration by endothelial cells: these cells allow them to transmigrate to lymph nodes, tumor lesions and infection sites. pDCs are also influenced by pro- or anti-inflammatory cytokines and growth factors produced by endothelial cells. For instance, endothelial cells produce IL-3 and VEGF that bind and trigger receptors on the pDCs' surface (CD123 and BDCA4 respectively). [²⁸] These bindings promote pDC migration and survival. Both resting and mature pDCs express ChemR23 (receptor for chemerin) on the surface. Chemerin is expressed on the surface of endothelial cells in the HEV and in the blood vessels of inflamed tissues. This interaction plays a role in pDC migration to lymph nodes and inflamed tissues. [²⁹]

T-cells

During infection, immature dendritic cells are recruited to the inflamed tissue. Upon pathogen sensing and activation by pro-inflammatory stimuli, they migrate towards the lymph node [³⁰]. The mature dendritic cells encounter naïve T-cells in the lymph node, and induce them to differentiate into effector T-cells [³¹, ³²]. The co-stimulatory molecules repertoire expressed and cytokines produced by DCs, differentially induced by the pathogenic stimuli, evoke a different immune response in each case. pDCs are not an exception: they influence T-cell polarization towards different subsets through different molecular interactions. pDCs are antigen-presenting cells (APCs): they are capable of presenting antigens on both MHC class I and II molecules, thus they can activate CD4⁺ T helper (Th) cells and CD8⁺ cytotoxic T cells. Due to their lack of co-stimulatory

molecules, resting pDCs induce T-cells anergy. Conversely, activated pDCs stimulate CD4⁺ T-cell polarization towards a variety of TH subsets (Th1, Th2, Th17, and Treg) [33].

pDCs express high levels of MHC molecules as well as co-stimulatory molecules such as CD80 (B7-1), CD86 (B7-2), CD40, OX40L and CD83 [34]. These molecules have a direct impact on TH polarization. According to the pathogen involved, pDCs can skew this polarization: virus-primed pDCs mostly induce a TH1 phenotype [35], while it is reported that IL-3- or TLR7-activated pDCs can induce, respectively, TH2 and TH17. pDCs can secrete Granzyme B that can affect the proliferation of T-cells. pDCs also express inducible T-cell co-stimulator ligand (ICOSL). The interaction of ICOSL with ICOS on T-cells has been shown to trigger naïve CD4⁺ T cells to produce IL-10. It has been suggested that this mechanism is important for generating Tregs to dampen the immune response, thus preventing excessive inflammation [36, 37]. pDCs also express programmed death receptor-ligand 1 (PD-L1); its interaction with programmed death ligand 1 (PD1) present on the T-cell surface can lead to anergy. IL-3 induces the expression of OX40L in pDCs; OX40L is a receptor associated with TH2 induction in T-cell polarization, with consequent release of IL-4, IL-5, and IL-13. Virus stimulation of pDCs can induce tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) that can induce apoptosis. TRAIL expression uniquely correlates with viral load and pDCs acquire the capacity to kill HIV-infected CD4⁺ T cells (a process called “TRAIL-dependent pDC-mediated killing”). [38]

There are other molecules expressed by pDCs that can influence T-cell function, for instance, lectin-like transcript 1 (LLT1). LLT1 is a ligand of CD161 (expressed by Th1, Th17, a subpopulation of CD8⁺ T cells, and NK cells); its binding leads to T-cell proliferation and IFN- γ secretion, and this inhibits NK cell cytotoxicity. [39]

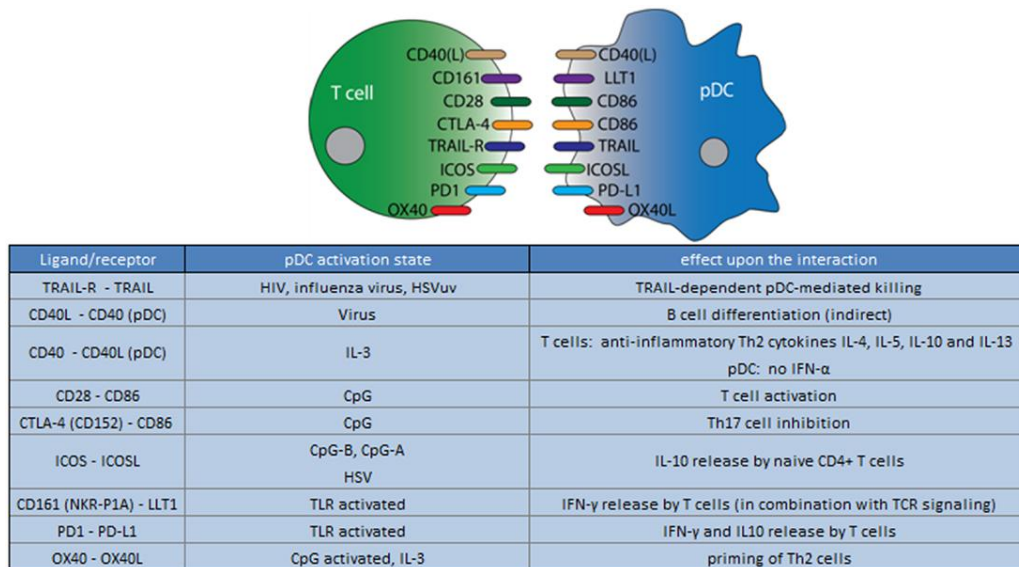


Figure 5: pDCs T-cell crosstalk. From Mathan et al., 2013

B-cells

B cells are the only cells capable of producing antibodies, and they play a critical role in the humoral response. Type I IFN induces an increase in TLR7 expression and activation markers on B-cells. IL-6 secretion by pDCs also plays a regulatory role on B-cells. Direct cell-to-cell interaction is also reported: CD40-CD40L and CD70/CD27 contact. These interactions result in B-cell growth, differentiation, and immunoglobulin secretion. Also, these interactions can be bi-directional, through LFA-1 or PECAM-1 (CD31), B cells stimulate Type I IFN secretion by pDCs. [40, 41]

Myeloid DCs

During inflammation, pDCs and mDCs are in contact *in vivo*, but this is also true at the steady state: could they act synergistically? The two DC subsets share many different functions, from the ability to present antigens to the expression of many co-stimulatory molecules. However, the two subsets are not redundant and each presents some unique features. These differences consist in the expression patterns of sensors (that will be discussed further below) but also in the production of cytokines and in the expression of co-stimulatory molecules. mDCs, for instance, are capable of

producing IL-1 β , IL-10 and IL-12p70 that pDCs do not secrete. In a viral infection, no other cells are able to secrete such high amounts of Type I IFN as pDCs and Granzyme B production is restricted to pDCs among the DC subsets [⁴²]. Other considerations suggest that pDCs and mDCs may cross-activate each other to reinforce the immune response. This crosstalk can be exerted by Type I IFN and TNF- α , but it seems there is a direct contact occurring. These interactions rely on CD40L/CD40, OX40L, HEVML, RANKL, CD27, CD30L, glucocorticoid-induced tumor necrosis factor receptor-ligand (GITRL), and 4-1BB. NOTCH ligands secretion by mDCs activates maturation markers on pDCs (such as CD86). After activation, pDCs express ICAM-1 (an adhesion molecule with immune stimulatory property) to match LFA-1 expression on mDCs. [⁴³]

NK cells and NKT cells

NK cells belong to the innate immune system and are capable of recognizing both virally infected and tumor cells due to the absence of MHC on the surface. They respond rapidly by lysing. Different studies highlight the crosstalk between pDCs and NK cells through direct and cytokine-mediated interactions. In tonsil T-cell areas, pDCs and NK cells are found in close proximity. During a Herpes simplex infection it was reported, that pDCs and NK cells migrated simultaneously to the infected site. pDCs and NK cells are also found in tumor lesions. These findings support the hypothesis that these cells have many possibilities to enter into contact. Type I IFN enhances the activation and the cytolytic potential of NK cells. pDCs express GITRL on the surface to bind GTR in NK cells. This binding enhances NK cell-mediated killing and their IFN- γ production. The CD69 NK cell surface expression is IFN- α and TNF- α dependent. Furthermore, direct contact between these two cell subsets leads to up-regulation of HLA-DR on the NK cells. HLA-DR expression on NK cells seems to exert a role in handling bacterial infection (shown for instance in *Mycobacterium bovis* (BCG) model). [⁴⁴]

This crosstalk is bi-directional, IL-2-stimulated NK cells induce pDCs to release type I IFN, also inducing up-regulation of CD83 in pDCs (but not CD80 and CD86) in a contact-dependent manner. Due to their high expression of MHC-I and lack of Nectin-2 (the ligand for NK cell activating receptors DNAM-1), pDCs are not susceptible to lysing; however, IL-3 stimulation can induce

Nectin-2 expression on mature pDCs. TLR stimulation induces LLT1, thus inhibiting the NK cells' cytolytic function, which saves pDCs from being killed. [⁴⁵]

Natural Killer T (NKT) cells are a T-cell subset expressing semi-invariant T-cell receptor (TCR- $\alpha\beta$) and surface antigens shared with NK cells. The TCR recognizes glycolipid antigens presented by the MHC class I-like molecule CD1d. iNKT cells are the best-characterized NKT subsets, as they express an invariant TCR- α chain that recognizes α -galactosylceramide (α -GalCer). Despite their lack of CD1d, pDCs crosstalk with iNKT cells directly, both via cell-to-cell interactions and by cytokine release. TNF- α and IFN- α release by pDCs enhance iNKT cell survival but not that of other NKT cell populations. pDCs interact with iNKT through the OX40L-OX40, augmenting iNKT IFN- γ release. Also pDCs cytokines augment iNKT responsiveness to CD1d mDCs presentation and induce TLR9 expression (otherwise they are unable to respond to CPG). [⁴⁶]

Tumor cells

pDCs infiltrate tumors: in fact, they have been found in different types of cancer lesions (melanoma, head and neck cancers, breast, ovarian and prostate cancers). pDCs, as stated before, express CCR6 (a receptor for skin homing, CCL20 ligand is produced by keratinocytes) and the chemokine receptor's expression level is higher in pDCs from melanoma patients compared to healthy volunteers. pDC infiltrates generally correlate with poor prognosis. pDCs infiltrating the tumor are generally in an immature state, suggesting a tolerogenic and immune-suppressive role. Prostaglandin 2 (PGE₂) and TGF- β seem to play a role in maintaining pDCs at an immature stage, inhibiting their secretion of IFN- α and TNF- α , and up-regulation of CCR7 (lymph node homing) [⁴⁷]. In this environment, pDCs can indirectly support tumor cell proliferation, migration, invasion and angiogenesis through release of IL-6 and IL-8. pDCs can also support tumor growth through the induction of Tregs; the majority of Treg cells express ICOS (pDCs, as stated before, can express high levels of ICOSL supporting Tregs expansion and suppressive function). Immature pDCs express immunoglobulin-like transcript 7 (ILT7) that is activated by bone marrow stromal cell antigen 2 (BST2, which can be produced by cancer cells): this binding leads to the inhibition of IFN- α and TNF- α production in response to TLR stimulation. [⁴⁸]

Properly activated pDCs behave differently: they induce anti-tumor T cell-mediated immune responses and kill cancer cells directly. Tumor cells are often TRAIL-sensitive: pDCs up-regulating TRAIL can induce apoptosis in these cells. ^[49]

On the other hand, tumors can exploit pDCs' ability in inducing a tolerogenic response by hijacking their immune strategy. GM-CSF has been reported to activate pDCs ^[50] and is found at high levels in breast cancer cell line supernatant (it has also been found in colon cancer and prostate cancer cell lines). Tumors also produce GM-CSF, and this has been confirmed in tumor supernatants and *in situ* through immunohistochemistry (IHC). The tumor-infiltrating pDCs express the receptor for GM-CSF and, once stimulated, induce production of TH2 cytokines (IL4, IL5, IL10, IL13, and TNF, but lower amounts of IFN- γ), suggesting a TH2 regulatory phenotype. It has been found that concomitant high levels of pDCs and GM-CSF are characteristic of aggressive breast cancers. In fact, the role of pDCs is the result of both the micro-environment they sense and the cells with which they communicate. ^[51]

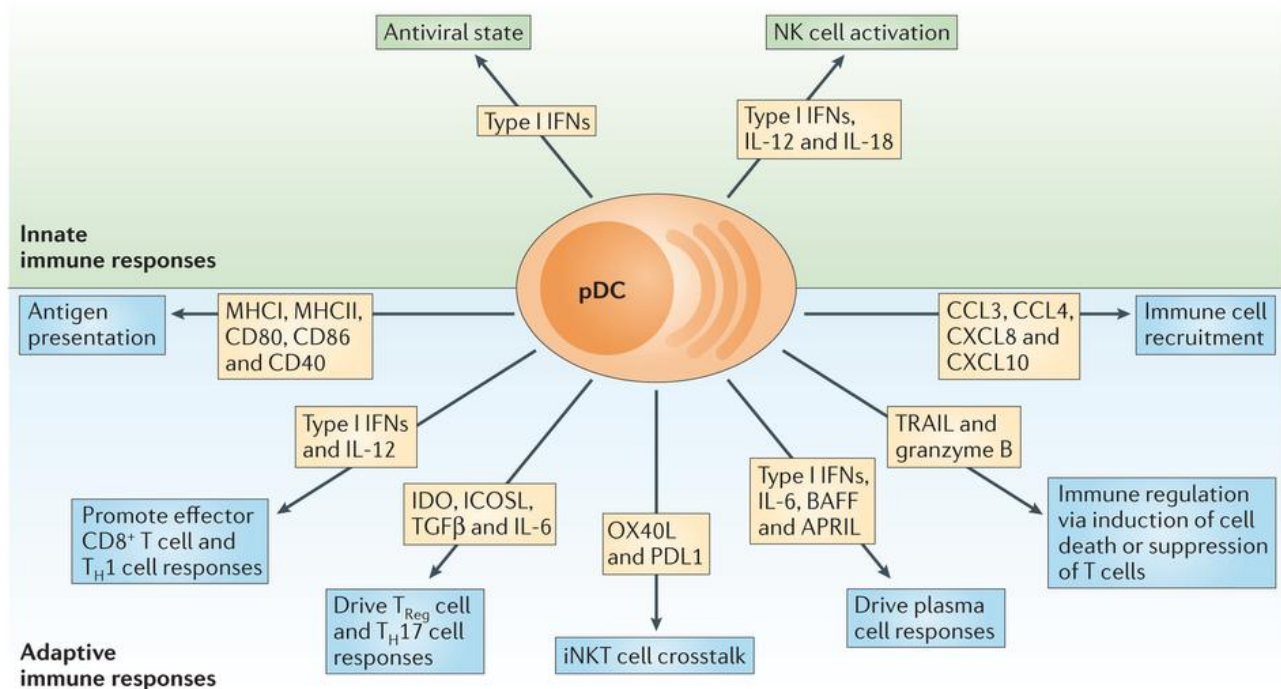


Figure 6: pDCs' crosstalk with the immune system. Swiecki et al., 2015

1.6 Dysregulation of the Plasmacytoid dendritic cell activation

pDCs are powerful and flexible cells: a dysregulation in their function can lead to a harmful immune response. There is multiple evidence that abnormal pDC activation leads to autoimmunity.

Patients with systemic lupus erythematosus (SLE) frequently have aberrant expression of genes downstream of type I IFN stimulation, and TLR7 and TLR9 (highly expressed by pDCs) play a role in (SLE). These findings suggest a possible role of pDCs in SLE [52]. As mentioned in the first chapter, E2-2 is a critical factor for the development of pDCs from bone marrow progenitors. Knock-out mice for E2-2 do not present pDCs *in vivo*; in the lupus model, mice have diminished autoantibodies against RNA, dsDNA and chromatin, suggesting pDCs' role in SLE [53]. Mice expressing the diphtheria toxin (DT) receptor (DTR) under the control of the *BDCA2* promoter show a transient pDC depletion after DT administration. Depletion of pDCs in lupus-prone mice results in reduced activation and expansion of the immune cells, restricted autoantibody production, and minimized kidney inflammation [54]. Moreover, pDCs are found in SLE lesions, tending to infiltrate the damaging tissues. Some studies suggest that pDC infiltration can be used as a diagnostic criterion. It is thought that one of the mechanisms through which pDCs impact SLE is through their high type I IFN production (Type I IFN receptor knockout leads to reduced autoantibody production) [55, 56]. The Type I IFN activates B-cells and it is one of the factors in the tolerance breaking. The germinal center (GC) is the place where antigen-reactive B cells expand and diversify. E2-2 mice have a reduced spontaneous GC reaction, fewer GCs and plasma cells, and an overall diminished GC-associated gene expression signature. Even if pDCs are able to sense nucleic acids, there are different mechanisms to prevent autoimmunity. Pathogens nucleic acids are delivered by endocytosis to TLR9 containing endosomes, and they contain unmethylated CpG motifs that activate TLR9. On the other hand, whereas self DNAs fail to access the endosomes, the high concentration of DNases in the extracellular environment degrades the self DNAs. Pathogens' DNAs

In lupus, the presence of self-DNA and DNA-specific antibodies complexes is considered the trigger for pDC activation (through the binding of the low-affinity Fc receptor to IgG (FcγRIIA; also known as CD32), followed by internalization and delivery to TLR9 containing endosomes [⁵⁷]. LL-37 is able to bind self-DNA to induce pDC TLR9 activation and Type I IFN release (although its role is better described in psoriasis, the LL-37 is among the most expressed genes and correlates with Type I IFN signature in LSE patients) [⁵⁸]. Moreover, HGMB1 (a nuclear DNA-binding protein released by dying cells) binds multimeric aggregated DNA complexes and binds to pDC RAGE receptor, facilitating the delivery of self-DNA to TLR9 [⁵⁹]. Self-RNA and auto-antibodies complexes can also activate pDCs, inducing a sustained Type I IFN production. Furthermore, terminally misfolded amyloid proteins interact with nucleic acids and these complexes activate pDCs. In mice, injection of amyloid DNA complexes or of bacterial amyloid–DNA complex induces the production of auto-antibodies [^{60, 61}].

It is hypothesized that multiple sclerosis (MS) is initiated by DC-induced activation of myelin-reactive T cells in the periphery. Activated CD4+ T cells migrate into the central nervous system (CNS), crossing the blood-brain barrier (BBB) and accumulating in the perivascular spaces where they are reactivated by resident APC (also like DCs). In the cerebrospinal fluid (CSF), and in the CNS of MS patients, accumulation of both myeloid DC and pDCs has been observed [⁶²]. Experimental autoimmune encephalomyelitis (EAE) mice models have confirmed the role of this DC subset in the pathology. In MS patients it has been observed that pDCs accumulate in the CSF, in white matter lesions and in the leptomeninges; moreover, pDC number is increased in CSF during MS exacerbations. Stimulated pDCs from MS patients present low levels of co-stimulatory molecules (CD40, CD83 and CD86) and a lower ability to stimulate T-cells priming and proliferation. pDCs have a low capacity to produce Type I IFN and show a reduced response to TLR7 antagonists. In some contexts, Type I IFN has a high tolerogenic potential; MS patients showed improved symptoms with IFN-β treatment. Type I IFN treatment in MS patients showed that pDCs augment the CD123 expression, and this could be linked to a major tolerogenic behavior (T-regs induction). [^{63, 64}]

Type I IFNs have also been implicated in the pathogenesis of Systemic sclerosis (SSc). Type I IFN is shown to induce SSc-like cutaneous sclerosis. PBMCs in SSc patients show increased Type I IFN-regulated genes signature. pDCs are also found in the lesions. Type I IFN also seems to play a role in Dermatomyositis (DM), a systemic autoimmune disease characterized by pathognomonic skin rashes and symmetric proximal muscle weakness. In DM there is a Type I IFN signature correlating with disease progression and, again, pDCs are found in the lesions. In Sjögren's syndrome (SS), another autoimmune disease of the connective tissue, up-regulation of the type I IFN pathway and an increase of pDCs in the lesions have been shown. It has been suggested that pDCs also play a role in vitiligo and in psoriasis, a chronic T-cell-mediated disease. pDCs infiltrate early psoriatic skin, and this correlates with the expression of markers typical of the early psoriasis phases. In mice models, blocking Type I IFN receptor or depleting pDCs improves the symptoms [⁶⁵, ⁶⁶]. In psoriasis, there is a large production of cationic anti-microbial peptides (AMPs). AMPs make up a family of peptides including cathelicidins and the defensins; these molecules are all amphiphatic or, rather, they contain a patch of positive charges clustered on one side. The AMPs bind to negatively-charged phospholipids, leading to the disruption of the microbial membrane. However, these peptides can bind other negatively-charged molecules, such as RNA and DNA fragments; this binding leads to the formation of aggregates protected from extracellular degradation. This complex can be endocytosed, and is recognized by TLR7 and TLR9. Activated pDCs seem to recruit pathogenic TH17 cells to the skin, and TH17 IL-17 secretion seems to activate a pathogenic loop inducing AMPs release by keratinocytes [⁶⁷]. Interestingly, pDCs were also found to be involved in Type I diabetes (T1D): an important role of Type I IFN in the initiation of the destructive autoimmunity was shown, and this correlated to the increased Type I IFN expression in pDCs in the pancreatic lymph nodes [⁶⁸, ⁶⁹].

In conclusion, this work presented the ability of pDCs to directly (through different surface molecules) or indirectly (through cytokine production) interact with many different cell types involved in the innate or the adaptive immune responses. These interactions shape or modulate the response of other cell types, showing that pDCs represent a knot in an intricate network. In this way, the activation of pDCs influences the innate and adaptive responses. pDCs aberrant

activation is involved in the local and systemic autoimmunity insurgence (it was hereby described a wide range of pathologies where there is altered pDCs activation: systemic lupus erythematosus, multiple sclerosis, systemic sclerosis, psoriasis etc...). These features show that pDCs are powerful cells, playing an important role in the immune system, and the relevance of deeply understanding how their behavior changes according to the environmental conditions.

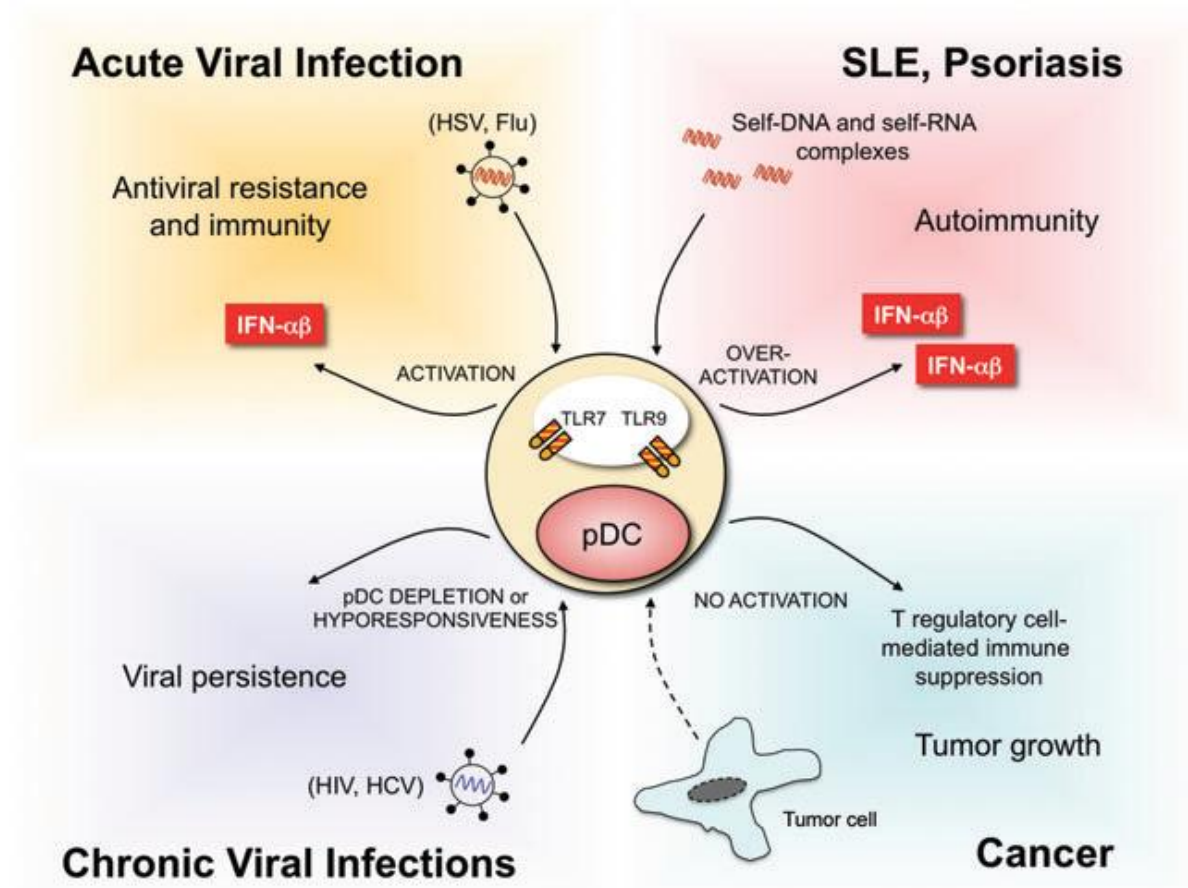


Figure 7. Involvement of pDCs in the human diseases. From Lande et al., 2010

2. Bacterial sensing

2.1 Gram positive bacterial infection

Bacteria are generally classified in two groups: GRAM+ and GRAM- Bacteria. GRAM+ bacteria become purple-coloured after GRAM staining while GRAM- bacteria appear red or pink. In this manuscript, I will focus on GRAM+ bacteria because they present a lipoprotein with a particular role in the pathogenesis on their surface. These lipoproteins are recognized by TLR2/1, and the role of TLR2/1 in pDCs is one of the questions of this thesis.

GRAM+ bacteria present different characteristics: cytoplasmic lipid membrane, thick peptidoglycan layer, lipoteichoic acids, rigid cell walls and a smaller volume of periplasm than that in GRAM- bacteria.

GRAM+ bacteria, comprehending both extracellular (for instance *S. aureus*) and intracellular (*L. monocytogenes*, *M. tuberculosis*) pathogens, developed different strategies to escape the immune system, many of them involving the exploitation of the immune sensors. GRAM These surface receptors are important in the recognition of extracellular bacteria. However, there are different mechanisms that allow engulfing the pathogen and permitting the activation of the intracellular receptors. In the same extent, intracellular pathogens also lead to the activation of surface receptors. Several studies highlighted their importance in the sensing process. In this chapter, I present the dendritic cells repertoire and a deeper characterization of TLR2 and TLR1, which consist the main object of this thesis.

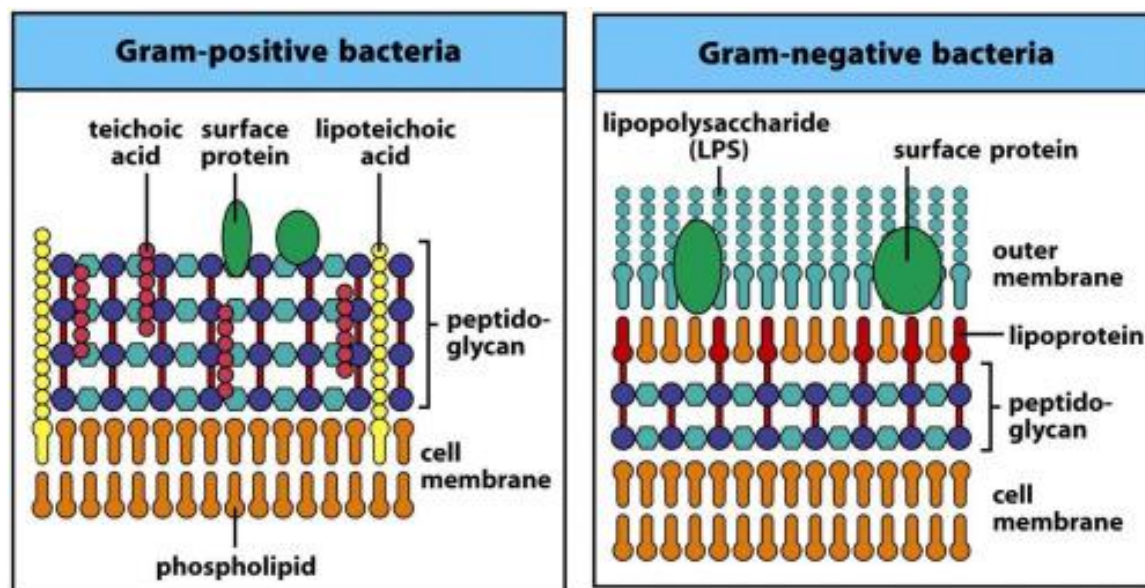


Figure 2-14 Immunobiology, 7ed. (© Garland Science 2008)

Figure 8. GRAM positive and negative bacterial membrane. From Janeway et al., 2008

S. aureus: a bacterium with two souls

S. aureus has been defined as “pathobiont”, a microbe that can switch from commensal state to a pathogenic state according to certain conditions (that are still unknown). *S. aureus* is the most common bacterium isolated from inpatient cultures and among the most common microbes from outpatient samples [70]. Moreover, *S. aureus* is considered the most potent skin pathogen; it colonizes about 30 to 50% of healthy adults intermittently and 10 to 20% persistently.

S. aureus can cause localized infections, like pneumonia or abscess, or a systemic risk like sepsis or toxic shock syndrome (TSS). In particular, it is a major risk for patients suffering from severe burns [71]. In 2005, 20,000 deaths and 500,000 hospitalizations cases were estimated in USA to be due to *S. aureus* infections. These numbers have grown during the last decade, due to the increased frequency of antibiotic multi-resistant strains. Despite an increase in mortality and morbidity, and *S. aureus* being considered a major health care problem, 25% of the population host this bacterium in the upper respiratory tract without developing any disease [72, 73]. Although large

cohort studies have been carried out, the genetic factors explaining why a quarter of the population chronically hosts *S. aureus* in the nasal swabs are not clear. [74]

S. aureus can induce TSS, an acute toxin-mediated illness caused by the expression of bacterial pyrogenic exotoxins called super-antigens (SAg). SAg binding to the β chains of the T cell receptor (TCR) in the T-cells and to the MHC-II on the DCs generates the over-activation of an enormous number of T-cells. [75] This over-activation causes what is called a “cytokine storm”, a massive release of cytokines with a TH1 profile characterized by high levels of interferon- γ (IFN- γ) and TNF- α . The patient suffers hemodynamic alterations, tissue ischemia that can lead to multi-organ failure, cardiovascular collapse, and death. Paradoxically, despite the large incidence of people carrying *S. aureus* in the nose, there is a remarkably low frequency of those developing TSS. Some groups have proposed that an evolutionary pressure has caused *S. aureus* to regulate its own pathogenicity. [76]

Listeria monocytogenes: the comet bacteria

Listeria monocytogenes is responsible for a severe food-borne disease characterized by meningitis, meningo-encephalitis, materno-fetal and perinatal infections. Generally, Listeriosis is considered an opportunistic infection in immune-compromised patients. *Listeria* bacteria cross the intestinal epithelial barrier, migrate through lymph and blood vessels to reach liver (they replicate inside hepatocytes) and spleen. Successively, the bacteria can disseminate in the brain and in the placenta, showing their capacity to cross the blood–brain barrier and the materno-fetal barrier. *Listeria* is capable of escaping neutralization by phagocytic cells and invading non-phagocytic cells.

Listeria bacteria enter in the cells through a “zipper” mechanism, whereby bacterial surface ligands interact with receptors in the host cells. The bacteria’s entrance into the cells is concluded with the pathogen entrapped in a sort of vacuole. In about 30 minutes, *Listeria* bacteria are able to escape from the vacuole and start to replicate in the cytosol. *Listeria* bacteria are also capable of recruiting actin and starting actin polymerization, which results in a network of branched

filaments. The polymerization process is located at one bacterial pole, allowing the bacteria to move in the cytoplasm at 10 μm per minute. *Lysteria* bacteria reach the membrane, inducing the formation of protrusions that allow them to invade neighboring cells and start a new infection cycle. This system protects the bacteria from the host's defenses. [77]

Resident macrophages are responsible for the initial killing of the bacteria by phagocytation and secretion of TNF- α and IL-12. NK cells are the initial source of IFN- γ , necessary to augment the macrophages' killing capacity. Innate immunity plays an important role in the initial control of infection, but T-cells are necessary for the final clearance of bacteria. CD4 TH1 and CD8+ memory cells are important for the protective response. [78]

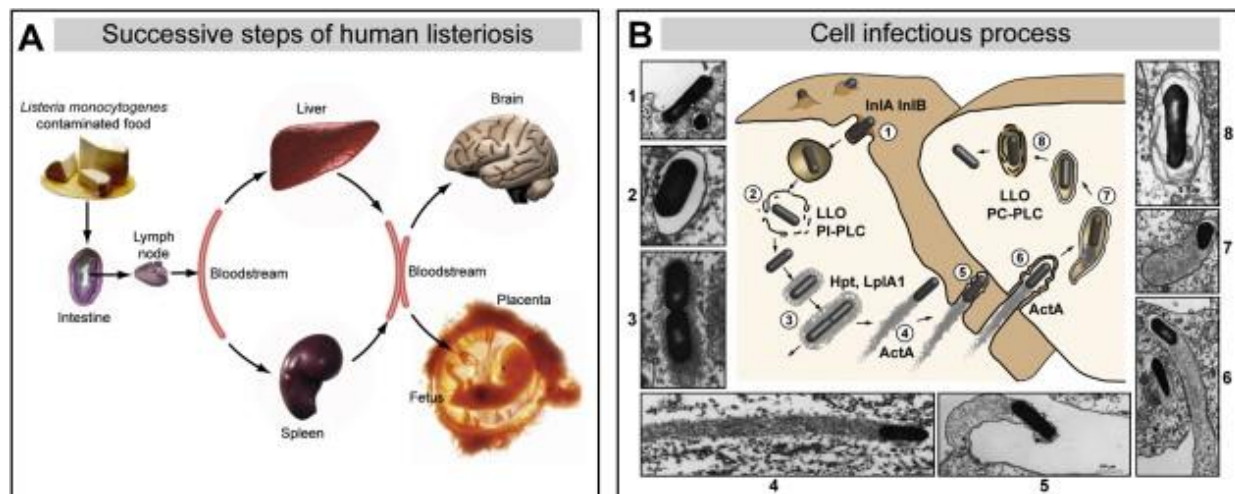


Figure 9: *Lysteria* model of infection **A.** Progression of *Lysteria* infection. **B.** Cell invasion process. Cossart et al., 2008

Mycobacterium tuberculosis: an old unfriendly companion.

Mycobacterium tuberculosis is an extremely successful pathogen that seems to have coevolved with the human species during an intimate relationship lasting thousands of years. This evolutionary success relies on the mycobacterial genome, designed to encode genes allowing

bacteria to escape the immune response. Moreover, mycobacterial strains resistant to antibiotics are increasingly widespread, constituting a serious threat for global human health. Tuberculosis causes approximately 1.5 million deaths per year: every year about 9 million new TB cases are diagnosed and it is estimated that 1/3 of the world's population is latently infected with *M. tuberculosis*. The only existing vaccine is the bacille Calmette-Guerin (BCG), shown to be protective only against the most severe disseminated form of the disease in children but with no effect in adult lung tuberculosis. MTB affects the lungs and is transmitted through aerosols produced during coughing and sneezing of patients with active disease. After its entrance in the lung, MTB is phagocytated by alveolar macrophages where it prevents its own active killing. MTB starts to replicate inside the macrophages and the phagosome. In most individuals, a delayed adaptive immune response is able to contain the pathogen in granulomas for a long time. In these heterogeneous structures consisting mainly of infected macrophages and T cells, *M. tuberculosis* encounters stress induced by reactive oxygen species and nutrient deprivation, which drives the bacilli into a latent stage. Resuscitation from this containment occurs upon disturbance of the immune system, such as HIV infection or a deficiency in IFN- γ or TNF- α production.

Inhibition of macrophage effector function and phagosome maturation: *M. tuberculosis* resides primarily in the macrophages, and bacilli use different sophisticated mechanisms to replicate and resist killing. In infected macrophages the fusion of phagosomes with lysosomes is abrogated (through avoidance of exposure to lysosomal hydrolases, low pH, and other components of lysosomes). The phagosomes fail to incorporate vacuolar ATPases, resulting in no acidification. New studies have found a serine/threonine kinase encoded by the *M. tuberculosis* *pknG* gene that is responsible for the inhibition of phagosome-lysosome fusion. [⁷⁹]

Prevention of macrophage autophagy: autophagy is a homeostatic process for eliminating damaged organelles; a double membrane forms an autophagic vacuole that engulfs organelles or unwanted structures within the cell. The vacuole is successively fused with the lysosomes. This

process can be induced by IFN- γ . Since this process can kill Mycobacteria, the bacteria express proteins to block it. [⁸⁰]

Infection and modulation of APCs: while macrophages are the most important site for intracellular replication, also DCs can be infected by Mycobacteria. Dendritic cells can present antigens, and prime a T-cells response against the pathogen. Dendritic cells infected by the bacteria are not sufficiently activated: they migrate to the lymph nodes and, instead of priming a protective immune response, they seem to help spread the bacteria to other tissues (as a kind of “Trojan horse”). Dendritic cells fail to mature in response to Mycobacteria infection, with reduced expression of MHC-II molecules on the surface and without a sufficient load of mycobacterial antigens. [⁸¹]

Modulation of the CD4 response: TH1 cells are crucial to a protective response, as their IFN- γ production stimulates the macrophages’ killing activity, leading to bacterial clearance. Despite some positive reports, the importance of the TH17 cells in the protective immunity development is still debated. By inducing reduced IL-12 production by monocytes and macrophages, Mycobacterium Tuberculosis affects TH1 development. [⁸²]

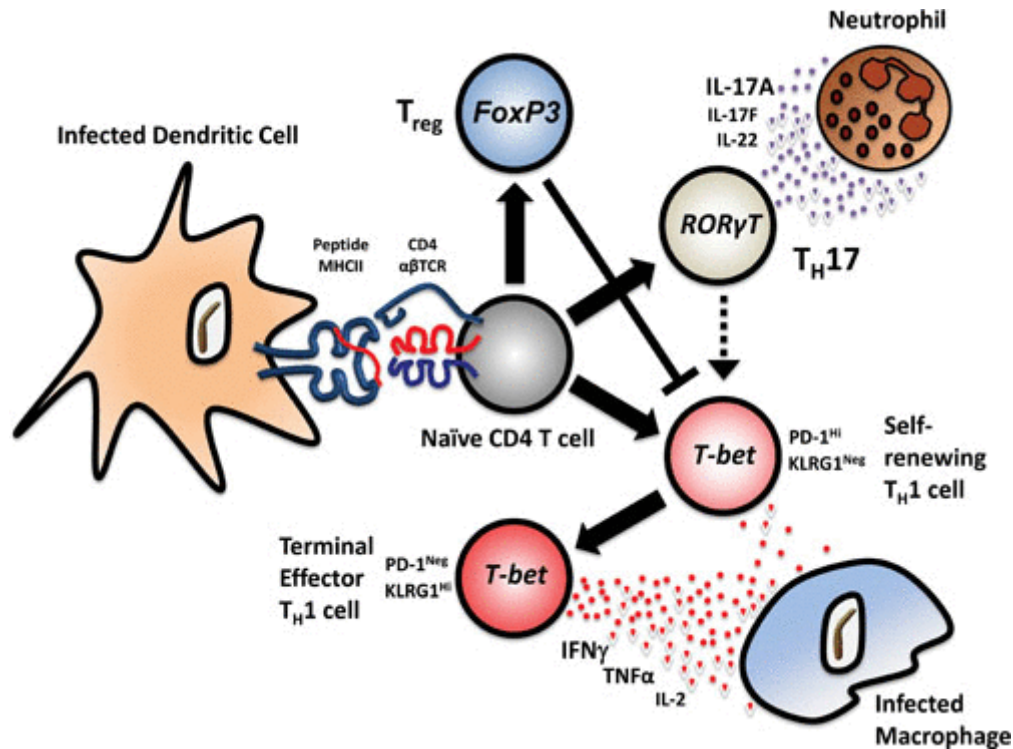


Figure 10: Infected DCs crosstalk with naïve CD4⁺. Goldberg et al., 2014

2.2 Innate immunity in GRAM⁺ bacterial infection

Pathological microorganisms enter the human body from different sites. These invasions are initially countered by innate immunity. Only when the innate host defenses are bypassed, evaded, or overwhelmed is an adaptive immune response required. Infections are generally rare, despite the fact that we are continuously exposed to potentially pathogenic agents, as many of them fail to overcome the epithelial barrier, and the ones that succeed to infiltrate themselves are in a great part eliminated. Epithelia are more than a simple physical barrier. Tears and saliva, for example, contain lysozyme (an antibacterial enzyme that hydrolyzes β -glycosidic linkages in the cell wall peptidoglycan), in the intestine antibacterial and antifungal peptides called cryptidins or α -defensins are secreted (by Paneth cells), in the respiratory and skin tract other antimicrobial peptides (β -defensins) are present. On the other hand, cationic peptides exist which are able to kill bacteria by damaging bacterial cell membrane. In the fluid coating the lung epithelial surfaces, two

proteins (surfactant proteins A and D) are present which bind to and cover the surfaces of bacteria, facilitating phagocytosis by macrophages. [⁸³]

During bacterial infection, phagocytic cells (macrophages and neutrophils) constitute the first line of defense against many common microorganisms and are essential for the control of common infections. The sensing of different pathogen by these cells leads to phagocytosis and successive killing of the microbe. However, infectious organisms cannot always be eliminated, as some bacteria have evolved their capacity to escape and others are not recognized. The lymphocytes in the adaptive immune system have evolved to provide a more versatile means of defense, increasing innate immunity capacity to kill and, in addition, providing increased protection against subsequent re-infection by the same pathogen. The cells of the innate immune system play a fundamental role in the initiation and subsequent guidance of adaptive immune responses, as well as the effective removal of pathogens targeted by an adaptive immune response. Moreover, as the adaptive immune response acts with a delay of 4-7 days, the innate immune response plays a critical role in controlling infection during this period [⁸⁴].

Like other pathogens penetrating the epithelial surfaces of the body for the first time, bacteria encounter cells and molecules that can activate innate immune response. Phagocytic macrophages and neutrophils express receptors on their surface, able to recognize and bind different constituents of common bacterial membranes. Activation through these receptors triggers the engulfment of bacteria by macrophages and leads to the release of biologically active molecules – cytokines and chemokines – which are a starting point for the inflammation.

Local inflammation and phagocytosis of invading bacteria may also be triggered by complement activation on the bacterial cell surface. Complement is a system of plasma proteins that, when binding on microbial surfaces (but not on host cells), initiate a cascade of proteolytic reactions. The coating of microbes with the resulting fragments leads to their recognition and binding by phagocytic receptors on macrophages. Complement allows phagocytic cells to recognize

pathogens that would otherwise escape phagocytosis and, thus, destruction. The process also releases small peptides that contribute to inflammation. Complement can be activated through three different pathways: binding C1q to the pathogen surface; mannan-binding lectin recognition of mannose on bacteria surface and consequent triggering of complement cascade; or spontaneous and stochastic activation of the cascade on the pathogen surface. Moreover, complement's final components are also able to damage bacteria by creating pores on their membranes. The membrane-attack complex has a hydrophobic external face and a hydrophilic internal channel. The diameter of this channel is about 100 Å, allowing the free passage of solutes and water across the lipid bilayer. The pores created by complement destroy the ionic balance and allow the entrance of lysozyme, leading to pathogen killing [⁸⁵]. Complement can be subverted by pathogenic bacteria, such as *S. Aureus*, expressing Staphylococcal protein A (SpA), an abundant cell wall-anchored surface protein. SpA binds tightly to the complement binding (Fcγ) portion of IgG and stimulates an abnormal B lymphocyte proliferation, provoking their clonal expansion and subsequent cell death [^{86, 87}].

Inflammation is characterized by increased local blood flow and fluid leakage. Chemokine attracts leukocytes. Among the first cells to migrate are the neutrophils (recruited in large number to the infected tissue), which possess different receptors for bacteria recognition and for their subsequent engulfing and destruction. Kinetically, monocytes concomitantly migrate or arrive a short time after neutrophils. Monocytes quickly differentiate into dendritic cells and macrophages.

Dendritic cells are also able to recognize and engulf bacteria (though less efficiently than phagocytic cells). This pathogen recognition leads to dendritic cells activation, maturation and migration to the lymph nodes, where they present the antigens to the T-cells. Dendritic cells provide all three signals necessary for T-cells activation: antigen presentation, co-stimulatory molecules, and cytokines.

Binding of many of the cell-surface receptors that recognize pathogens leads to bacteria being phagocytated. In this active process, the pathogen is surrounded by the phagocytic cell membrane, and thus internalized in a membrane-bounded vesicle known as a phagosome. This latter undergoes different maturation steps during an increasingly acidified process: on one hand, vacuolar ATPase are recruited, leading to a pH of 5.5-6; on the other hand, there is a fusion of phagosomes with lysosomes, (granules containing enzymes, proteins, and peptides involved in an intracellular antimicrobial response). The fusion of phagosome and lysosome leads to a new structure called phagolysosome, where bacteria are actively killed [⁸⁸]. In addition, macrophages and neutrophils produce other toxic substances to kill the engulfed bacteria, such as: hydrogen peroxide (H_2O_2), the superoxide anion (O_2^-), and nitric oxide (NO), which are directly toxic to bacteria. These products are generated by lysosomal NADPH oxidases that transfer an electron from cytosolic NADPH to molecular oxygen. Superoxide (O_2^-) can dismutate to form hydrogen peroxide (H_2O_2), which in turn can react with superoxide to generate singlet oxygen (O_2^\bullet) or hydroxyl radicals (collectively defined as reactive oxygen species (ROS)). Superoxide and hydrogen peroxide damage enzymes; and H_2O_2 reacts to cysteine residues, while hydroxyl radicals damage DNA. Different pathogenic bacteria have developed different mechanisms to neutralize these toxic products. In certain circumstances, macrophages express Myeloperoxidase (MPO), which converts superoxide into hypochlorous acid and chloramines, other potent products toxic to bacteria. This microbial activity is tightly regulated to avoid damaging tissue (oxygen reactive species are also able to damage host tissues) and is generally activated by the receptors' pathogen recognition. Macrophages generate nitric oxide (NO) by using inducible nitric oxide synthase (NOS2). The NO is produced in the cytoplasmatic side of the phagosome and diffused inside. NO reacts with ROS to form peroxynitrite and nitrosothiols that can lead to protein inactivation, lipid oxidation, and DNA damage [⁸⁹]. These two mechanisms are regulated and increased by the IFN- γ produced by TH1 T-cells. The NO production is important in bacterial clearance of *S. Aureus* infections, as demonstrated by the fact that patients deficient in NOS2 undergo severe diseases. Macrophages also express other proteins with antimicrobial activity in the phagosome. These proteins sequester critical nutrients for bacterial growth (divalent cations like Fe^{2+} , Zn^{2+} , and Mn^{2+}). This process, called Nutritional Immunity, is also important in bacteria killing, as many

pathogens rely on their hosts' resources [90]. During phagosomal maturation, natural resistance-associated macrophage protein 1 (NRAMP-1) depletes Fe- and Mn- from the phagosome lumen [91]. On the contrary, the lumen is enriched with Cu and Zn ions (macrophages express Zn and Cu transport proteins such as Znt1 and ATP7A). These metal ions are toxic to several bacteria that do not possess detox enzymes. In addition, phagocytes contain different proteases with the potential to digest bacteria: endopeptidases (cysteine and aspartate proteases), exopeptidases (serine and cysteine proteases), hydrolases that degrade carbohydrates (for instance lysozyme) and lipids (phospholipase A2) [92]. Neutrophils express serine proteases (elastase and cathepsin G) [93]. Macrophage elastase (Matrix metalloprotease (MMP) 12) is a protease delivered to the phagolysosome with the role of killing Gram+ and Gram- bacteria [94]. MMP12 is important in the early killing of *S. Aureus*, as it adheres to the bacterial cell wall and disrupts its membrane. Many of these enzymes need a low pH to be activated, and the acidified pH also inhibits microbial growth. When the canonical phagolysosomal killing mechanisms are exhausted, macrophages undergo apoptosis as further mechanism to kill a high load of bacteria. Neutrophils (but a similar mechanism has been proposed to macrophages and other cells) release neutrophil extracellular traps (NETs) through a process called NETosis [95]. During this process, which leads to neutrophil death, they eject their chromatin into the extracellular milieu (enriched with antimicrobial proteins including, defensins, myeloperoxidase, lactoferrin and calprotectin) with the intent to entrap and subsequently kill bacteria. For instance, *S. Aureus* is sensible to these NETs, but pathogenic *S. aureus* have been found able to secrete nuclease enzymes and degrade NETs [96]. Some pathogenic *S. Aureus* strains are able to escape from the phagosome (they express α -hemolysin (Hla)) and, moreover, block macrophages apoptosis (this mechanism seems crucial to fighting the intracellular persistence of living bacteria). In addition, some strains express different proteins that can block phagocytosis.

ROS and NO are potently toxic products that can seriously damage the host tissues: in fact, cells exposed to ROS form adenosine, a potent immunosuppressive signaling molecule. Pathogenic *S. aureus* expresses adenosine synthase A (AdsA), which converts adenosine monophosphate (AMP) to adenosine, a molecule that inhibits neutrophil superoxide bursts or degranulation, and also

leads to loss of T-cell adherence and an increased production of IL-10. Some strains express superoxide dismutases (SodA and SodM), catalase (KatA), alkyl hydroperoxide reductase (AhpCF), and staphyloxanthin, that protects or mitigates the damage induced by ROS [97]. They express urease to degrade urea into ammonia (weak base), to contrast acidification.

S. aureus pathogenic strains modify their surface membrane (they express a cell envelope modification system that attaches D-alanine to cell wall teichoic acids) to reduce the bacterial surface's negative charge and limit the microbe's susceptibility to cationic antimicrobial peptides (AMPs). This system inhibits another killing mechanism produced by macrophages and neutrophils (as alpha-defensins). In addition, *S. aureus* becomes insensible to lysozyme, which is largely present in the neutrophils granules, by modifying its peptidoglycan. Successful pathogens like *S. aureus* or MTB have developed nutrient acquisition systems that can circumvent the host's nutritional immunity response.

Alveolar macrophages are among the first host cells to encounter Mtb in the lungs following aerosol transmission. [98] They represent a major cellular niche for bacterial replication during the initial phase of the infection and serve as reservoirs for bacteria persistence within the lung granulomas during the chronic phase. MTB reside in the non-acidified phagosomes, reverting the functionality of macrophages. The macrophages response to IFN- γ activates an antimicrobial killing program that allows MTB clearance. [99]

The most abundant cell type found in the bronchoalveolar lavage (BAL) and sputum of active pulmonary TB patients is neutrophils: within the lungs, they are only second to lymphocytes [100]. Neutrophils play a dual role in tuberculosis, mediating antimicrobial activity but also leading to host damage. This is due to their release of granules containing elastase, collagenase, and myeloperoxidase [101]. During chronic infection, neutrophils can express the programmed death ligand 1 (PD-L1) with an immune regulatory effect. [102]

Macrophages and dendritic cells release cytokines , like IL-6, TNF and IL-1, which are also called pyrogens, because they induce fever (the temperature increase is believed to reduce bacterial replication). These cytokines induce hepatocytes to produce acute-phase proteins. These proteins, like C-reactive protein and Mannan-binding lectin, are released into the blood stream and are thought to bind the pathogens, as well as facilitate complement activation and phagocytic engulfment. These cytokines and Type I IFN also activate NK cells in order to kill intracellular bacteria.

Dendritic cells have been shown to constitute a significant population of cells harboring MTB *in vitro* and *in vivo* (studies conducted in mice models). Whether dendritic cells serve as a major cellular niche for MTB replication *in vivo* remains unclear. Dendritic cells, as previously explained, play an important role in bridging innate to adaptive responses. In TB, immature DCs are impaired in their function and prone to induce a tolerogenic response with induction of IL-10 and blockade of the protective response. Mature DCs induce a TH1 response with consequent IFN- γ release, fundamental for the activation of phagocytic cells antimicrobial killing. For instance, during MTB infection, the binding of DCs DCSIGN receptor to MTB ManLAM leads to the induction of IL-10 release, which is implicated in the impairment of DC maturation and expression of co-stimulatory molecules. Different reports indicate that MTB modulates DC functions in order to prevent optimal induction of host adaptive immunity. The impaired ability of DCs to stimulate T cells is also reflected on the infection late phase, with compromised CD4+ and CD8+ memory response.

Natural killer cells, granular innate lymphocytes possessing potent cytolytic capacity, are also part of the innate immunity. NK cells act early during an infection. Their activation is MHC-restricted, but dependent on the engagement of a number of their activating cell surface receptors to ligands upregulated by stressed or infected target cells. In the TB infection context it has been found that various MTB cell-wall components (such as mycolic acids) are direct ligands for the natural cytotoxicity receptor (NCR) NKp44 on NK cells. These cells are able to kill infected macrophages and produce IFN- γ and IL-22, which can inhibit intracellular growth of MTB *in vitro* by enhancing

phagolysosomal fusion [103]. In TB, NK cells are impaired, as patients with pulmonary TB display decreased frequencies of NK cell subsets and lower expression of IFN- γ .

2.3 Dendritic cells sensors repertoire

DCs play a sentinel's role in the immune system; they migrate in the peripheral and as well in the inflamed tissues to sense the menace. Pathogen recognition is mediated by various receptors which, through different pathways, induce DC maturation. DCs incur in a number of changes during this maturation process: for example, they express different co-stimulatory molecules and secrete different cytokines. The ensemble of these inputs shapes the T cells' prime and final outcome of the response. The differential pattern of co-stimulatory molecules and cytokines is the result of the downstream signals derived by the different receptors' activations. In this frame, the importance of the variety of these sensors is clearly understandable.

In vertebrates, sensing the pathogens relies on two systems:

- *Pathogen-recognition receptors (PRRs)*: sensors of this class recognize the molecules of the pathogen; there is a direct binding of these molecules which is defined as pathogen-associated molecular patterns (PAMPs)).
- *Danger-associated receptors*: during the infection the damage of the host tissues leads to the release of danger-associated molecular patterns (DAMPs).

Cytosolic RNA sensors

The RIG-like helicases (RLH) are a family of RNA sensors which includes three members: retinoic acid-inducible gene-I (RIG-I), melanoma differentiation antigen 5 (MDA5), and RLH3. Moreover, new helicases implied in cytosolic RNA sensing have been discovered: DDX1, DDX21 and DHX36. These helicases unfold the RNAs and with their CARD domain interact with adaptor proteins. The signaling passes through MAVS converging in the Type I IFN induction and NF- κ B activation with

subsequent pro-inflammatory cytokines release. Generally, these sensors respond to virus infection or to pathogens that are escaping TLR sensing. [¹⁰⁴]

Cytosolic DNA sensors

In the last few years different cytosolic DNA sensors have been identified whose signaling passes through TBK1 and IRF3, inducing Type I IFN secretion. Different genes belong to this class of sensors, for instance:

- *ZBP1*: the first identified cytosolic DNA sensor capable of binding the DNA z-form.
- *LRRFIP1*: identified in mice macrophages responding to *Listeria monocytogenes* and binding the DNA in a leucine-rich repeat domain.
- AIM2-like receptors (ALRs): a family of four genes capable of binding the DNA and signaling through inflammasome and leading to IL-1 β production. It is reported that non-virulent mycobacterial strains induce IL-1 β production followed by AIM2 activation.
- *DDX41*: once activated, it interacts with STING to induce Type I IFN production.
- *cGAS*: the latest identified sensor, presents a enzymatic activity after DNA binding to activate, in turn, STING and the Type I IFN production. In *Chlamydia trachomatis* and *Listeria monocytogenes* infections it seems that cGAS plays a role in IFN- β production.

Other cytosolic DNA sensors are present and, most likely, further ones will be identified in the next few years. This redundancy shows the danger associated to the DNA presence in the cytosol.

C-type lectin receptors

C-type lectin receptors (CLR) are a family of plasma membrane proteins that recognize conserved carbohydrate moieties from pathogens. These receptors contain carbohydrate recognition domains binding carbohydrate structures in a calcium(Ca²⁺)-dependent manner. C-type lectins are either present as transmembrane proteins or secreted as soluble proteins. [¹⁰⁵]

- Mannose receptor: it recognizes mannose or fucose on the bacterial surface; it is one of the principal receptors mediating macrophages phagocytosis. It can recognize LAM and ManLAM from the *Mycobacterium Tuberculosis* cell wall. The engagement of the Mannose receptor in macrophages triggers the production of IL-10, IL-1R antagonist and IL-1R type II, which dampen the immune response. Despite the apparent detrimental effect on the host, mice deficient in this receptor do not show more resistance to mycobacterial infections. [¹⁰⁶]
- *DC-SIGN*: it is a type II transmembrane receptor that recognizes mannan, expressed on macrophages and on some DCs subsets. In *Mycobacterium Tuberculosis* it recognizes mannosyl residues of LAM, although its role in the mycobacterial infection is unclear (also because mouse homologs do not recognize the same pathogen moieties), DC-SIGN promoter polymorphisms have been associated with an increased risk of tuberculosis in South African cohorts; results, however, are not confirmed in other cohorts. [¹⁰⁷, ¹⁰⁸, ¹⁰⁹]
- *Dectin-1*: Dectin-1 is a type II transmembrane receptor that recognizes β -glucans and which is expressed on DCs and neutrophils. What it recognizes on *Mycobacterium Tuberculosis* is unknown. What is known is the role of its activation in IL-1, IL-23, TNF, and IL-6 production by monocytes derived dendritic cells leading to TH1/TH17 T-cells priming Dectin-1 $-/-$ mice do not show significant differences in inflammatory cytokine levels during the mycobacterial infection: this and other evidence suggest a possible redundant role. [¹¹⁰]
- *Mincle*: it is an inducible C-type lectin receptor that recognizes trehalose-6,6' dimycolate (TDM) from *Mycobacterium Tuberculosis* and is expressed on macrophages. After binding, it induces pro-inflammatory cytokines. [¹¹¹]

Scavenger receptors

Scavenger receptors can be expressed on the cell surface or secreted from monocytes and macrophages. They are capable of recognizing different ligands: self and non-self lipoproteins.

- *CD36*: CD36 is expressed on different cell types; it recognizes and internalizes oxidized low-density lipoproteins (LDL). In the innate immunity frame, this sensor recognizes bacterial diglycerides including lipoteichoic acid (LTA) from Gram-positive bacteria. The binding induces pro inflammatory cytokines such as TNF- α . [¹¹²]
- *MARCO*: MARCO is expressed by macrophages and is up-regulated during bacterial infection. It recognizes LDL and TDM; its activation leads to NF κ B signaling and pro-inflammatory cytokines release. [¹¹³]
- *Scavenger receptor A*: this receptor exerts the ability to bind and internalize endogenous proteins and microbial ligands (for example LPS from Gram-negative bacteria and LTA from Gram-positive bacteria). It can associate with TLRs to enhance the endocytosis of microbes. Its impact in mycobacterial infections is still debated. [¹¹⁴]
- *AIM*: it is a receptor secreted by tissue macrophages and promotes macrophage survival. It seems to provide a protective role in mycobacterial infection by promoting cathelicidin production. [¹¹⁵]

CD14

CD14 binds LPS from Gram-negative bacteria and lipoteichoic acids, and peptidoglycan from Gram-positive bacteria. It is expressed on monocytes and macrophages and is involved in phagocytosis. In the mycobacterial infection it recognizes LAM. [¹¹⁶]

NOD receptors

NODs are a cytoplasmic family of over 20 receptors. The best-known, NOD1 and NOD2, recognize bacterial cell wall peptidoglycan; their binding to the ligands leads to NF κ B and consequent pro-inflammatory cytokines production (as TNF, IL-12p40, RANTES). NOD-deficient mice are more susceptible to mycobacterial infection; they succumb sooner in comparison with wild-type. Knock-down in human macrophages augments the bacterial load in them. [¹¹⁷]

NLRP3

NLR family, pyrin domain-containing 3 (NLRP3) is important in the inflammasome formation; this multi-protein complex leads to the activation of caspase-11. Caspase-11 is important in the cleavage of pro-IL-1 β and pro-IL-18; once cleaved, these two cytokines can be secreted. IL-1 β plays a protective role in mycobacterial infection. The specific NLRP3 still remains elusive and NLRP3 $-/-$ mice show no great differences in survival and control of the infection. [¹¹⁸]

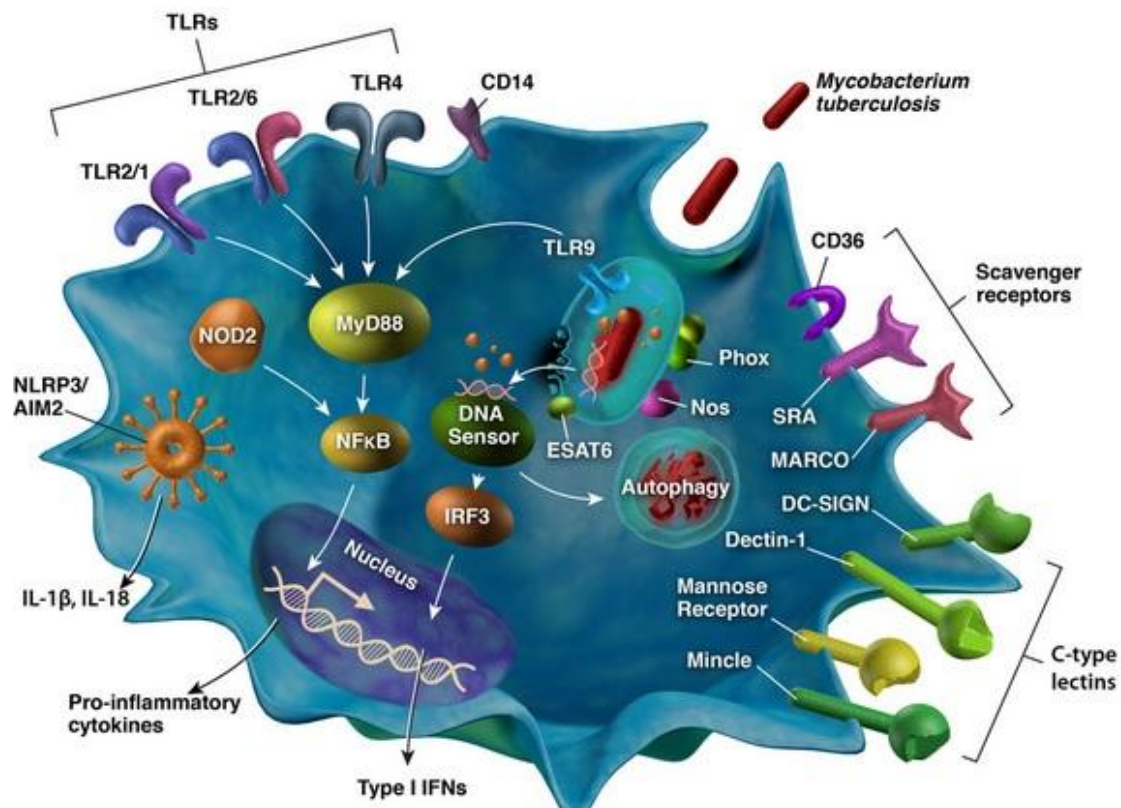


Figure 11: The different sensors present in dendritic cells. From Stamm et al., 2015.

2.4 Toll-Like Receptors

Toll-like receptors (TLR) are the first family of PRRs to have been characterized; they recognize different types of molecules from pathogens. TLRs consist, respectively, of 10 and 13 members in humans and mice. TLR1-10 are conserved in the genome of the two species, while TLR10 locus is disrupted in the mice genome by insertion of a retrovirus. In the human genome TLR11, TLR12 and TLR13 are not present. TLRs differentially recognize lipoproteins, lipids and nucleic acids thanks to the various N-terminal outer membranous domains called leucine-rich repeat (LRR). TLRs are composed of a transmembrane domain and the C-terminus is involved in signal transduction (Toll–interleukin 1 receptor (TIR) domain). [¹¹⁹]

TLRs are divided into cell surface type and endosome type. In humans, TLR1, TLR2, TLR4, TLR5, TLR6 and TLR10 are located on the surface, whilst TLR3, TLR7, TLR8 and TLR9 are in the endosome. Cell-surface type TLRs mainly recognize microbial components present in the membranes of the bacteria or fungi (lipoproteins, lipids and proteins). Conversely, endosomal TLRs recognize nucleic acids from pathogens (bacteria or virus).

TLR2 recognizes various molecules of pathogens including bacteria, viruses, fungi and parasites. TLR2 forms heterodimers with TLR1 or TLR6 to differentially recognize triacyl and diacyl lipoproteins. TLR2 and TLR1 will be examined more in detail below.

TLR5 recognizes a bacterial flagella-derived protein called flagellin. TLR5 is highly expressed on intestinal lamina propria dendritic cells; it plays a role in the CD4 T-cell priming towards TH17 and in the B-cells IgA secretion. [¹²⁰]

TLR4 plays an essential role in the recognition of lipopolysaccharide (LPS). LPS represents the main GRAM- bacteria cell wall component and is the major causative of lethal septic shock. CD14 with LPS-binding protein (LBP), a soluble plasma protein, bind and deliver the LPS to TLR4. TLR4 forms a complex with MD2: while the phosphate groups of LPS interact with positively charged residues

of TLR4, the LPS lipid chains are bound by a hydrophobic portion of MD2. TLR4 is also capable to detect viral envelope or fusion proteins from viruses. TLR4 is able to sense its ligands either on the cell surface or in the endosomes. [¹²¹]

TLR3 is located in the endosomes and recognizes double-stranded RNA (dsRNA), believed to be generated in some virus replication (such as West Nile virus, respiratory syncytial virus and encephalomyocarditis virus). TLR3-mutated humans show to be highly susceptible to herpes simplex virus type 1 (HSV-1). Studies performed in vitro with the synthetic dsRNA analog, polyinosinic–polycytidylic acid (poly(I:C)), show that TLR3 macrophages and DCs result in strong type I IFN secretion (although poly(I:C) in dendritic cells can induce Type I IFN in a TLR independent pathway through RIG-I). Structural Analysis performed on the crystal structure of the extracellular domain of TLR3 bound to dsRNA showed that the N-terminal domain of TLR3 is a horseshoe-like shape thought to increase the surface area that can interact with dsRNA. [¹²²]

TLR7 is another endosomal TLR that recognizes single-stranded forms of RNA (ssRNA). TLR7 has been shown to recognize ssRNA from human immunodeficiency virus, influenza virus and vesicular stomatitis virus. Different synthetic ligands are available, such as poly(U) RNA, and nucleic acid analogs, such as imiquimod and resiquimod (R-848). This last compound can also activate TLR8, another receptor that recognizes ssRNA. TLR7 activation leads to pro-inflammatory cytokine production and Type I IFN. TLR7 and TLR8 are tandemly aligned and share highly similar aminoacid sequences. TLR8 is expressed in monocytes, macrophages and myeloid dendritic cells. [¹²³, ¹²⁴]

TLR9 is yet another endosomal TLR. It recognizes unmethylated CpG DNA, which is highly frequent in bacteria, viruses and parasites, but rare in mammalian cells. Malaria parasites produce DNA and hemozoin (this is the residual product of heme-detoxification by Plasmodium). Granulin, an unusual cysteine-rich protein, can interact with CpG DNA and facilitate the binding with TLR9. [¹²⁵]

TLR10 is predominantly expressed in immune cell-rich tissues, such as the small intestine, stomach, thymus, peripheral blood lymphocytes, lymph nodes and tonsils. Co-immunoprecipitation studies have shown the association of TLR10 in homodimers or in heterodimers with TLR1 and TLR2: these interactions are likely to be mediated by the extracellular domain of these receptors. For a long time the ligand specificity of TLR10 has remained elusive, but bioinformatic analysis suggests that the ligand could be lipoproteins. [¹²⁶]

The endosomal localization of TLR3, TLR7, TLR8 and TLR9 is determined by their transmembrane region. This localization is important for avoiding autoimmune response against unmethylated CpG self-derived DNA [¹²⁷]. UNC93B1 associates with the transmembrane domains of endosomal TLRs and helps them translocate from the endoplasmic reticulum (ER) to the endosomes, where they sense their ligands. There are other proteins involved for the endosome localization, such as gp96 and PRAT4A. Lack of these proteins show hyporesponsiveness in mice. To recognize its ligand, TLR9 has to be cleaved into the N-terminus by intracellular peptidases. After the LPS recognition, TLR4 is internalized by a clathrin-/dynamin-dependent mechanism and directed to the endosomes. [¹²⁸]

MyD88-dependent pathway

MyD88 interacts through its death domain with the kinase IRAK4 which, in turn, interacts with IRAK2. The complex MyD88–IRAK4–IRAK2 interacts with E3 ubiquitin ligase and TRAF6. The binding of TRAF6 with TAB2 and TAB3 activates MAP kinase cascade. Moreover TRAF6 transfers K63-linked polyubiquitin to the regulatory subunit of I κ B kinase (IKK) complex called NEMO (also known as IKK γ). IKK is phosphorylated by TAK1; following this event, IKK phosphorylates I κ B proteins, bound to NF- κ B subunits to prevent its nuclear translocation. After its phosphorylation, I κ B is degraded and the remaining NF- κ B subunits (p50 and p65) translocate to the nucleus, leading to the production of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6 and IL-12 p40). The timing of individual gene expression for these cytokines is differentially regulated by MyD88-dependent pathways. The TNF- α and IL-1 β mRNAs are transcribed at early time points. Conversely, IL-6 and IL-12 p40 mRNAs are up-regulated later. [¹²⁹]

TRIF-dependent pathway

TRIF recruits IKK ϵ and TBK1, and the latter phosphorylates IRF3. After phosphorylation, IRF3 translocates into the nucleus and activates a strong Type I IFN production. TLR3-mediated NF- κ B activation is critically dependent on TRIF; in TLR4 activation, instead, both MyD88 and TRIF are necessary for NF- κ B translocation. NF- κ B activation is mediated by the binding of TRAF6 and RIP1/RIP3 to TRIF. [¹³⁰]

2.5 Toll-Like Receptor 2 and Toll-Like Receptor 1

Toll-Like Receptor 2 and Toll-Like Receptor 1 can heterodimerize. TLR2, however, is better characterized and can heterodimerize with numerous partners. The crystal structure of the TLR2/1 and TLR2/6 heterodimers have been characterized. In the binding of TLR1/2 heterodimer with the synthetic triacylated lipopeptide Pam₃CSK₄ (structural analog of lipoprotein), it was observed that the 2 glycerol-bound lipid chains of Pam₃CSK₄ bind with the hydrophobic pocket of TLR2 and the

third amide-bound lipid chain docked on TLR1: this process therefore leads to a stable structure. TLR2 in combination with TLR6 can bind different lipoproteins. The resolution of the crystal structure with the diacylated lipopeptide Pam₂CSK₄ shows that diacylated lipopeptide is docked on TLR2 and the lipid chains are docked on the exposed hydrophobic surface on TLR6. This heterodimer is less stable, and is reinforced by several strong hydrogen bonds between the two TLRs. TLR10 is a member of the TLR1/2/6/10 cluster and it has been hypothesized that it can play a role as TLR1 or TLR6. Co-immunoprecipitation studies show the presence of heterodimer with TLR1 and TLR2; moreover, bioinformatic analysis shows that the structure of TLR1/TLR10 and TLR2/TLR10 is likely to be stable. During ligand-induced TLR10 binding assays the binding of triacylated lipopeptides to TLR10 was observed. [¹³¹]

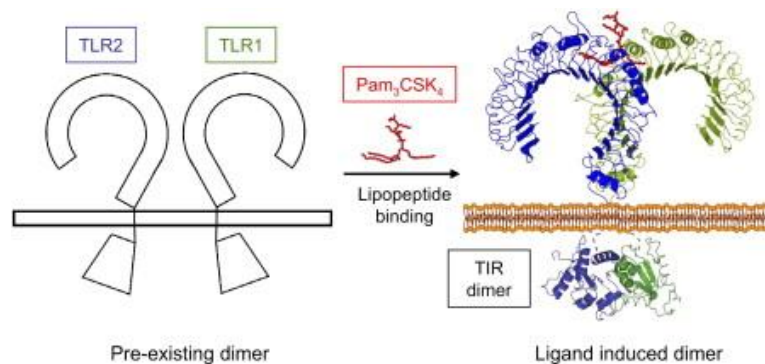


Figure 13: Model of the Ligand-Induced Heterodimer of Full-Length TLR1 and TLR2. Jin et al., 2007.

These heterodimers preexist on the surface (this is confirmed by fluorescence resonance energy transfer (FRET) in monocytes) but their increase is directly related to the presence of the ligand. In absence of ligands, these heterodimers are unstable and their associations are only temporal. Some studies suggest that heterodimers migrate to lipid rafts upon the ligand binding. The disruption of the lipid rafts has showed to impair activation. Lipid rafts are “membrane islands” with a different composition in lipids: there are highly ordered saturated lipids and a major

content of cholesterol. These islands are sparse in the more fluid membrane (higher content of unsaturated lipids). After cells activation, lipid rafts islands cluster to form a large platform. Receptors and adaptor molecules accumulate in the lipid rafts during activation. [¹³²]

TLR2 can also homodimerize: in this case it is able to recognize microbial membrane components such as lipoteichoic acids (LTA) and peptidoglycan (PGN). TLR2 can also recognize some endogenous ligands that occur in response to an inflammation. These ligands play a role as DAMPs. Here is a list of some of them (other have been proposed but whether they are really ligands or not is still being debated) [¹³³].

- Pancreatic adenocarcinoma up-regulated factor (PAUF)
- Amyloid β (A β): it has been established that synthetic peptide aggregates of A β 1-42 are recognized by TLR2 and its activation is enhanced with TLR2/1 heterodimers.
- α -Synuclein: TLR2 binds the α -Synuclein when this molecule is in oligomer form and adapted to a β -sheet conformation.
- Serum amyloid A (SAA)
- SNAP-associated protein (Snapin)
- β 2-Glycoprotein I (β 2-gpI)

TLR2 can heterodimerize with other receptors as, for instance, CD14 (CD14 has also been showed to interact with TLR3, TLR4, TLR7 and TLR9). CD14 directly binds TLR2 on the membrane surface. With the use of surface plasmon resonance techniques, CD14 has been found to increase the responsiveness of TLR2/1 heterodimers to triacylated lipopeptides. CD14 is highly concentrated inside lipid rafts as a glycosylphosphatidylinositol-anchored membrane protein; this causes TLR2/1 heterodimers to migrate to lipid rafts. [¹³⁴]

TLR2 can also interact with integrins, even if only few studies have reported direct interaction. Integrins are generally heterodimeric receptors that exert the role in cell-cell and cell-extracellular

matrix interactions. Integrins activation leads to changes in the membrane phosphoinositide composition: this attracts some specific TLR adaptor molecules to the lipid rafts, thus leading to enhanced TLR activation. Interestingly, the integrin subunit β_3 interacts directly with TLR2 in resting monocytes; the stimulation with a synthetic triacylated lipopeptide induced a rapid dissociation of this complex. The integrin β_3 binds vitronectin: this soluble protein that is generally present in the blood or in the extracellular matrix has the capacity to opsonize microbes. In this frame, it has been suggested that integrin β_3 concentrates the ligands to TLR2/1 to enhance activation. [¹³⁵]

TLR2 can also heterodimerize with Dectin-1, leading to activation in response to zymosan (fungal membrane component) and some *Mycobacterium* species.

There is high evidence showing that lipoproteins (TLR2/1 ligands) play an important role in TLR2 activation by staphylococci bacteria. The lipoproteins are abundant in GRAM + bacteria, representing about 2–3% of the bacterial proteome. Their biosynthesis is a multistep process requiring a high-energy effort for the bacteria. These proteins are translated within the cytoplasm and later modified at the membrane level. The mutants in different genes, important for the lipoprotein maturation of various *S. aureus* strains, are severely affected in pathogenicity [¹³⁶]. This is also true for different GRAM+ pathogens with impaired growth and pathogenicity. In MTB, the *IspA* mutant (gene for LPP maturation) is markedly attenuated in virulence, showing decreased intracellular multiplication in mouse macrophages and growth decrease in lungs and spleens of mice. Reductions in the pathogenicity have also been noticed in *Streptococcus pneumoniae* (severely affected in growth, virulence, adherence, and the oxidative stress response), *Streptococcus pyogenes*, and in *Listeria monocytogenes* (the mutant was impaired in invasion and intracellular survival, showed to be attenuated in a mouse infection model, was unable to induce TLR2 activation and exhibited increased susceptibility to cationic peptides) [¹³⁷]. In the two main groups of Gram-positive endospore-forming bacteria, LPP turned out to be crucial to spore germination and spore formation. *Bacillus anthracis* LPP mutants showed decreased innate

immune stimulation and were affected in spore germination in both *in vitro* and mouse infection models, showing markedly decreased virulence [¹³⁸]. Moreover, in *Clostridium difficile*, which causes severe gastrointestinal diseases, the LPP have been found to be essential to sporulation and transmission [¹³⁹]. These elements explain the evolutionary importance of bacteria, and that of relative sensing by the host. In *S. aureus* around 55-70 different lipoproteins are present, some of which only in particular strains. Around 50% of the LPP were annotated as transporters for amino acids, peptides, iron, zinc, or molybdenum or as chaperones, but still many of the proposed LPP showed no similarity to known proteins, and their function awaits elucidation [¹⁴⁰]. LPP proved important for *S. Aureus* to invade keratinocytes and mouse skin. Also triacylated lipoproteins are present in *S. aureus*. TLR2-deficient macrophages release significant lower amounts of pro-inflammatory cytokines in response to *S. aureus*. TLRs are not phagocytic receptors *per se* but undergo internalization and can facilitate bacteria engulfment. It is reported that TLR2 (and other TLRs) accelerate the phagosome's maturation. Moreover, bacterial engulfment can lead to degradation of the bacterial cell wall, making more ligands available for TLR2 recognition, which amplifies the response. Neutrophils play an important role in the immune response to *S. aureus*: neutropenia or neutrophil dysfunction result in recurrent staphylococcal diseases. TLR2/1 are expressed by neutrophils; Pam₃CSK₄ activation induces secretion of pro-inflammatory cytokines (IL-8, MIP-1 α , MIP-1 β , MIP-3 α , and IL-1 β). In a model of *Staphylococcus*-induced mice septicemia TLR2 has been shown to help enhance survival [¹⁴¹]. These elements explain the evolutionary importance of the LPP for bacteria, as well as the importance of relative sensing by the host. [¹⁴²]

In MTB, around 99 putative lipoproteins have been found encoded in the genome. LPP form an important part of the surface component, localized not only in the plasma membrane or in the mycobacterial outer membrane; in fact, some of them are even shed from the membrane. LPP are important for nutrient uptake, drug export, cell wall homeostasis and direct host-pathogen interaction through host cell adhesion. Many of them have shown to be glycosylated; the purpose of this modification, however, remains unknown. TLR2/1 is responsible for recognizing different components of the mycobacterial membrane. The relationship between them and the above TLRs is problematic, as with *Mycobacterium tuberculosis* it can be either beneficial or detrimental. TLR2

activation through Rv0577 (MTB complex-restricted secreted protein) leads to DCs maturation with co-stimulatory expression and pro-inflammatory cytokine release through MyD88-, MAPK- and NF- κ B-dependent pathway activation. In mice, Rv0577-treated DCs induce TH1 and TH17 phenotype, and TLR2 $-/-$ mouse models have fewer TH17 cells in the lung. Lipomannan (a TLR2 ligand) from several mycobacterial species is able to trigger macrophage activation with the production of TNF- α and IL-6. *M. tuberculosis* 19 kDa lipoprotein is a TLR2/1 agonist and their binding leads the heterodimers to translocate to lipid rafts. After this translocation, the production of reactive oxygen species (ROS) in macrophages is induced. TLR2 can recognize mycobacterial lipoglycoproteins, but causes neither strong activation nor subsequent T-cells priming. *Mycobacterium indicus pranii* (MIP), a non-pathogenic mycobacterium recognized by TLR2, has been proposed as a vaccine candidate to protect against tuberculosis and leprosy. Moreover, *Mycobacterium abscessus* (MAB), recognized by TLR2 (while the TLR2 and Dectin-1 lead to the internalization in macrophages), has emerged as an opportunist pathogen in bronchiectasis patients, but can potentially lead to severe and even lethal lung infections. [¹⁴³]

MTB infections can induce macrophage death via apoptosis or necrosis (apoptosis appears to be protective, while necrosis promotes bacterial growth and dissemination, and enhances damages to the surrounding tissues). Apoptosis depends on the synergy of TLR2 and TLR4 signals, while the TLR2 signal alone is responsible for necrosis. Mycobacterial lipoprotein LpqH can induce antibacterial autophagy through TLR2/1 pathway [¹⁴⁴]. In human monocytes, activation of TLR2/1 induces Vitamin D production, which plays an antimicrobial role. TLR2/1 activation also induces IL-1 β secretion and the up-regulation of IL-1 receptor; conversely, there is a down-regulation of the IL-1 receptor antagonist. [¹⁴⁵]

During allergic diseases and helminth infections there is an increased presence of eosinophils in the peripheral blood and tissue. In mycobacterial infections there is an enhanced recruitment of eosinophils within the lung granuloma. Lipomannan induces the eosinophil activation through TLR2. NK cells can also be activated by TLR2 mycobacterial ligands.

The above evidence leads to conclude that TLR2 and TLR2/1 play a beneficial role for the host, except that MTB can hijack TLR2 for immune evasion. One of the facts that can explain the outstanding evolutionary success of MTB is its own capacity of modulating its gene expression according to the different infection stages. Initial MTB-expressed genes promote DCs activation but subsequently expressed genes can block DCs activation. MTB express genes to down-regulate MHC class I and II on infected macrophages. Further studies show that MTB genes enhance the TLR2-mediated expression of suppressors of cytokine signaling 1 in DCs, leading to reduce IL-12 secretion and block ROS production [¹⁴⁶]. Suppressors of cytokine signaling 1 lead to the decrease of inducible NO synthase 2 expression in macrophages. Pathogenic mycobacteria block the macrophage activation and phagosome maturation via TLR2, and lead to induce IL-10 production. Virulent mycobacteria contain proline-proline-glutamic (PPE) motifs, PPE18 (one of the PPE family) specifically interacted with TLR2; this interaction leads an early and sustained activation of p38 MAPK in macrophages, followed by IL-10 production [¹⁴⁷]. MTB heat shock protein 60 (hsp60) interacts with TLR2, inhibiting the nuclear c-rel transcription factor with consequently IL-12 p40 secretion decrease and favoring TH2 skewing in the response. [¹⁴⁸]

Bacteria naturally secrete vesicles (called membrane vesicles (MVs)), which contain toxins and immune-modulatory molecules. *M. tuberculosis* and BCG produce MVs in a virulent related manner. Proteomic analysis has shown that only MVs released by virulent strains contain TLR2 lipoprotein agonists. When injected in the lungs of mice, these MVs induce inflammatory responses in a TLR2-dependent manner. On the other hand, if they are administered to mice lungs before MTB pulmonary exposure, an increase in the bacterial replication is observed. [¹⁴⁹]

All these elements show the importance of lipoproteins in GRAM+ bacterial pathogenicity and their possible participation in the pathogens overcome of the immune system. As reported in this chapter, TLR2 KO mice succumb quickly to GRAM+ bacterial infection. It would be a too simplistic view to consider TLR2 important for bacteria sensing. *M. Tuberculosis* and other pathogenic bacteria evolved to hijack the immune system, being TLR2 and the other co-receptors part of the

escape strategy.. How the interplay among these TLRs works is still not fully understood, but it seems likely that they play a role in the balance between mounting a protective response and an immune evasion.

2.6 pDCs sensing

The different Dendritic cells subsets express different PRR. [150] pDCs express different receptors on the surface and in the cytosol that allow them to sense microbes in the surrounding environment. pDCs express TLR7 and TLR9 at high levels, which will be discussed in detail below. pDCs also express RIG-I, DHX9 and DHX36 to sense viral replication in the cytosol, cGAS, C-type lectin receptors as BDCA-2, DEC-205 and DCIR. Moreover, they express FC receptors on the surface (FcγRIIIa, FcαR and FcεRI have been reported to be expressed by pDCs): these receptors are important for the uptake of microbes complexed with antibodies, and their activation induces the mobilization of MHC-II molecules from lysosomal vesicles to the plasma membrane.

TLR7 and TLR9 mediated sensing

Among the DCs subsets, pDCs have been described to be important in viral sensing and immune response for their unique capacity to produce an extraordinary amount of Type I IFN in viral infections. This capacity relies on high expression of TLR7 and TLR9 and their internal machinery. The engagement of TLR7 and TLR9 receptors leads to a sustained Type I IFN production. The role of Type I IFN in bacterial infection and the fact that the TLR7/9 pathway is partly shared with TLR2/1, make an interesting issue for discussion. Whether or not pDCs express TLR2 and TLR1 will be discussed in the results.

As stated above, TLR7 and TLR9 reside in the endosomes. TLR7 and TLR9 undergo post-translational maturation in the ER, the chaperone protein gp96 plays a role in these modifications and, following these steps, UNC93B helps translocation from ER to endosomes [151]. TLR7 recognizes guanosine- and uridine-rich single-stranded RNA, while TLR9 recognizes phosphodiester backbone in natural DNA or unmethylated CpG [152, 153]. The binding of the ligands

with these TLRs lead to the activation and translocations of NF- κ B and IRF7; these molecules, however, are recruited at different time points. In the early endosomes, TLR7 and TLR9 activation leads to recruitment of MyD88 with the subsequent association of IRAK4 and TRAF6, Bruton's tyrosine kinase (BTK) and IRF7. IRF7 is the master regulator transcription factor for the pDCs Type I IFN production [¹⁵⁴, ¹⁵⁵]. The assembly of this complex is responsible for the IRF7 phosphorylation. Phosphatidylinositol 3-kinase (PI3K) activates AKT that is phosphorylating IRF7 [¹⁵⁶]. Phosphorylated IRF7 translocates in the nucleus, where the transcription of Type I IFN genes begins. IRF7 is constitutively highly expressed in pDCs; this allows the rapid recruitment and assembly of the multimeric complex defined above. Moreover, pDCs lack the translational repressor 4E-BP and they too constitutively express high levels of IRF8 (thus permitting an amplification of the Type I IFN production). Interestingly, another mechanism grants pDCs to be efficient in the Type I IFN production because they retain DNA complex in the early endosome for an extended period of time with continuous activation of IRF7. IRF7 positively regulates its own expression, creating a positive amplification loop. [¹⁵⁷]

Conversely, maturation in the late endosome provides a different signaling pathway. TLR7 and TLR9 also recruit MyD88, BTK, TRAF6 and IRAK4 but, in these cases, triggering the ubiquitinylation of the protein kinase transforms growth factor- β (TGF- β)-activated kinase 1 (TAK1). TAK1, as described above, is important in phosphorylation and degradation of I κ B. These events lead to NF- κ B activation and translocation and the activation of MAP kinases cascade. These two signaling pathways are responsible for co-stimulatory expression and pro-inflammatory cytokines production (TNF- α and IL-6). [¹⁵⁸]

Type I IFN has a potent effect on the immune system and, generally, on the body. For these reasons production of pDCs IFN has to be under tight control. Due to excessive secretion, an aberrant Type I IFN production by pDCs can seriously harm the host. There are different surface receptors modulating this production: for instance, BDCA2 and ILT-7 associate with Fc ϵ RI [¹⁵⁹, ¹⁶⁰]. The ITAM motif is involved in the suppression of the pDCs' ability of produce IFN in response to

TLRs activation. In the same way, FcεR1α associates with FcεR1γ and NKp44, leading to the recruitment of DAP12 that bears an ITAM motif. Other receptors that modulate Type I IFN are CD300A and CD300C, DCIR, BST2 and leukocyte-associated immunoglobulin-like receptor 1 (LAIR1). It is reported that VEGF increases the Type I IFN. In addition, TGF-β and PGE2 inhibit the Type I IFN production through SMAD activation. [¹⁶¹, ¹⁶²]

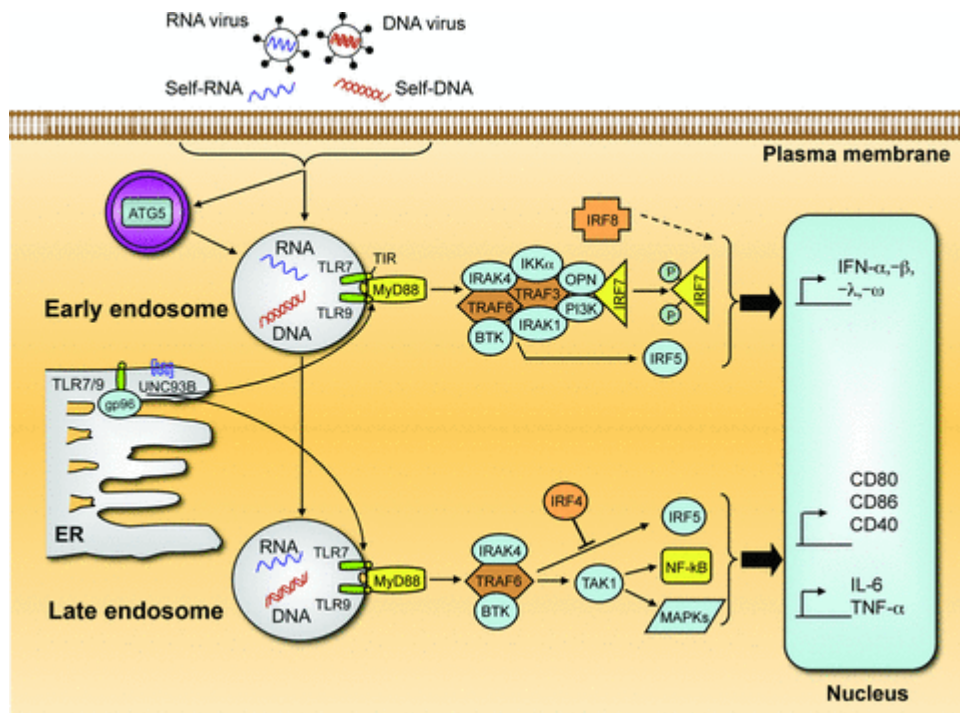


Figure 14: Spatiotemporal differential signaling of TLR7 and TLR9 in pDCs. From Lande et al., 2010.

TLR	Location of TLR	PAMPs and DAMPs recognized by TLR	Co-receptor or (s)	Signalin g adaptor	TF	Effector cytokines induced

TLR1/2	Plasma membrane (cell surface)	Triacyl lipopeptides (Bacteria and Mycobacteria). Amyloid β	TLR1/2	MyD88	NF κ B	Inflammatory cytokines (TNF- α , IL-6 etc.)
TLR2	Plasma membrane (cell surface)	Peptidoglycan (Gram-positive bacteria), LAM (Mycobacteria), Hemagglutinin (Measles virus), phospholipomannan (<i>Candida</i>), Glycosylphosphatidylinositol mucin (<i>Trypanosoma</i>) Heat shock proteins, Necrotic cells, Urate crystals, PAUF, α -Synuclein, β 2-Glycoprotein I, Serum amyloid A	CD36, CD 14, integrins, dectin-1	MyD88	NF κ B	Inflammatory cytokines (TNF- α , IL-6 etc.)
TLR3	Endosome	ssRNA virus (WNV), dsRNA virus(Reovirus), RSV, MCMV		TRIF	NF κ B, IRF3, 7	Inflammatory cytokines (TNF- α , IL-6 etc.), type I IFNs
TLR4	Plasma membrane (cell surface)	LPS (Gram-negative bacteria), Mannan (<i>Candida</i>), Glycoinositolphospholipids (<i>Trypanosoma</i>), Envelope proteins (RSV and MMTV). Heat shock proteins, HMGB1, Extravascular fibrinogen/fibrin, Lung surfactant protein, Minimally modified LDL, Pancreatic elastase	MD2, CD14, LBP, RP105	MyD88, TRAM and TRIF	NF κ B, IRF3, 7	Inflammatory cytokines (TNF- α , IL-6 etc.), type I IFNs

TLR5	Plasma membrane (cell surface)	Flagellin (Flagellated bacteria)		MyD88	NFκB	Inflammatory cytokines (TNF-α, IL-6 etc.)
TLR6/2	Plasma membrane (cell surface)	Diacyl lipopeptides (Mycoplasma), LTA (Streptococcus), Zymosan (<i>Saccharomyces</i>)	Heterodimer of TLR6/2	MyD88	NFκB	Inflammatory cytokines (TNF-α, IL-6 etc.)
TLR7	Endosome	ssRNA viruses (VSV, Influenza virus)		MyD88	NFκB, IRF7	Inflammatory cytokines (TNF-α, IL-6 etc.), type I IFNs
TLR8	Endosome	ssRNA from RNA virus		MyD88	NFκB, IRF7	Inflammatory cytokines (TNF-α, IL-6 etc.), type I IFNs
TLR9	Endosome	dsDNA viruses (HSV, MCMV), CpG motifs from bacteria and viruses, Hemozoin (<i>Plasmodium</i>)		MyD88	NFκB, IRF7	Inflammatory cytokines (TNF-α, IL-6 etc.), type I IFNs
TLR10/1 or 2	Plasma membrane (cell surface)	Bacterial lipoprotein		MyD88	NFκB	Inflammatory cytokines (TNF-α, IL-6 etc.)

	surface)					IL-6 etc.)
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Table 1. Toll-like receptors summary. Adapted from Kumar et al., 2009.

3. Plasmacytoid dendritic cells and bacterial infectious disease

3.1. Type I Interferon: a friend or a foe?

The interferon genes family has been divided into three distinct groups. In humans, the type I IFN family encodes 13 partially homologous IFN α subtypes, IFN β and some other genes that are poorly characterized (IFN ϵ , IFN τ , IFN κ , IFN ω , IFN δ and IFN ζ) [¹⁶³]. IFN- γ is the only gene that belongs to the type II IFN family, and is mainly produced by T-cells and natural killer cells [¹⁶⁴]. The last group defined as type III IFN family encodes IFN λ 1, IFN λ 2 and IFN λ 3 (also known as IL-29, IL-28A and IL-28B, respectively) [¹⁶⁵]. The role exerted by the Type III IFN family looks similar to the first group but with a less broad activity since the receptor's expression is restricted to epithelial cells.

Type I IFN family is well known because of its important role in viral infections, while a massive release of IFN α/β induces a general antiviral state in the cells. IFN α/β can be released by infected and uninfected cells and, after binding the receptor, induces a broad transcription gene program to contrast the viral infection.

Almost any cells in the human body can secrete type I IFN in response to the stimulation of different receptors that recognize microbial products, known as pattern recognition receptors (PRRs), to be discussed in detail further below. There are different receptors capable of inducing the Type I IFN production, which exploit different pathways (depending on cell type) lately converging into the IFN-regulatory factor (IRF) family. These master regulator genes play a key role after translocating from the cytoplasm to the nucleus, thus powerfully inducing IFN α/β transcription [¹⁶⁶].

IFN α/β binds to a heterodimeric transmembrane receptor composed of two subunits: IFNAR1 and IFNAR2. The binding of the cytokine with the receptor triggers two associated kinases (Janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2)). These are phosphorylating STAT1 and STAT2, both

present in the cytosol; in turn, they dimerize and translocate into the nucleus to bind to IRF9 to form the ISG factor 3 (ISGF3) complex. This complex is binding to IFN-stimulated response elements in ISG promoters, leading to the activation of ISG transcription [¹⁶⁷]. There is also an alternative pathway passing through other STAT protein as STAT3, STAT4 and STAT5.

The binding IFN α/β induces the transcription of hundreds of genes, stimulating broad antiviral program in the cell. The name Interferon derives from its ability to interfere with the viral replication in vertebrate cells. This effect is mediated by the large number of antiviral genes induced in the cells [¹⁶⁸]. Some genes acting against ISGs are present in different virus genomes and this demonstrates the importance of this antiviral program in contrasting the infection [¹⁶⁹]. Here, as an example, is a small list of the best-known ISGs:

- Myxovirus resistance 1 (MX1)
- IFN-inducible double-stranded RNA-dependent protein kinase (PKR; encoded by EIF2AK2)
- 2'-5'-oligoadenylate synthetase (OAS),
- IFN-induced transmembrane proteins (IFITMs)
- Apolipoprotein B mRNA-editing enzyme catalytic polypeptide 1 (APOBEC1)
- Tripartite motif-containing (TRIM) family

The use of exogenous IFN α/β to treat viral infections in mouse models has suggested a role in the antiviral immunity. The knockout of the IFN receptor in mice has proved that Type I IFN has a pivotal role in the protection against the virus *in vivo*. The absence of receptors was shown to be impacting the outcome in different viral infection models. In humans, a naturally occurred mutation in the JAK and STAT genes has been found, thus providing further evidence. [¹⁷⁰, ¹⁷¹, ¹⁷²],. The role of Type I IFN in the resolution of the infection is less clear in other contexts: studies in rhesus macaques during simian immunodeficiency virus (SIV) transmission and acute infection have shown the importance of timing in IFN administration. Because of the extent of the powerful immune response and wide gene program induced, the results lead to consider that type IFN

makes the system extremely complex. Alterations of this delicate equilibrium can change the balance from a therapeutic response to detrimental consequences. [¹⁷³]

Ligand	Alternate names	Human gene locus	Receptor chain 1	Receptor chain 2	Signal transduction pathways
Type I IFN	IFN- α	9p21+3 (T)	IFN- α R1	IFN- α R2	Jak1, Tyk2
	IFN- β	9p21+3 (T)			Stat1, Stat2, Stat3
	IFN- δ	None			Stat4, Stat5
	IFN- ϵ	9p21+3 (C)			PI3K
	IFN- κ	9p21+1 (T)			Akt
	IFN- τ	None			NF κ B
	IFN- ω	9p21+3 (T)			MAPK
	Limitin	None			p53 PRMT1
Type II	IFN- γ	12q14+3 (C)	IFN- γ R1	IFN- γ R2	Jak1, Jak2 Stat1, Stat3, Stat5

Ligand	Alternate names	Human gene locus	Receptor chain 1	Receptor chain 2	Signal transduction pathways
IFN					PI3K Akt NF κ B MAPK
Type III IFN	IFN- λ 2 (IL-28A)	19q13+2 (T)	IL-28R1	IL-10R2	Jak1, Tyk2
	IFN- λ 3 (IL-28B)	19q13+2 (C)			Stat1, Stat2, Stat3
	IFN- λ 1 (IL-29)	19q13+2 (T)			Stat5

Table 2. Type I, II, III family members. Adapted from Pestka et al., 2004.

The effects of Type I IFN go far beyond the induction of an antiviral program in the cells, as its secretion has an impact on the entire immune system: IFN α/β affects myeloid cells, B cells, T cells and NK cells supporting the immune response and their activation in the frame of resolving the infection and stimulating the generation of memory. Therefore, there is new evidence of its detrimental role under certain conditions where IFN α/β can trigger immune suppression or lead to tissue damage when exacerbating immune activation.

Abundant evidence from mice and human studies shows IFN α/β to promote precursor differentiation in the dendritic cells. IFN α/β stimulation on immature DCs enhances their expression of MHC-II complex, CD80 and CD86, thus stimulating their capacity to cross present antigens to CD8⁺ T-cells and their migration through the lymph nodes (up-regulation of chemokine receptors), inducing IL-12 (fundamental for the TH1 polarization) [¹⁷⁴, ¹⁷⁵, ¹⁷⁶, ¹⁷⁷, ¹⁷⁸, ¹⁷⁹, ¹⁸⁰]. Despite this beneficial role in mouse influenza, high levels of IFN α/β and pDCs were found in susceptible strains. pDCs and IFN α/β were leading to high inflammation and lung damage, resulting in poor survival. The pathogenic mechanism was identified in the TNF-related apoptosis-inducing ligand (TRAIL) by pDCs and its death receptor 5 (DR5) expressed by epithelial cells. [¹⁸¹]

Type I IFN also acts directly on CD4⁺ and CD8⁺ T-cells and, with context-dependent activity, promotes or inhibits T-cell activation, survival, proliferation cytokine production (IFN- γ as an example), cytotoxic function and memory formation. In humans, IFN α/β is associated with a TH1 polarization in CD4⁺ T-cells that play a protective role in different viral infections. IFN α/β supports the memory T-cell effector function and trafficking during a secondary infection. Conversely, in HIV infection IFN α/β induces chronic inflammation and facilitates CD4⁺ recruitment and HIV spread. IFN α/β can promote T-cells death through the TRAIL and DR5 expression. One study showed that in HIV infection Type I IFN mediates the TRAIL expression by pDCs, enabling these cells to induce TRAIL-dependent CD4⁺ T cell apoptosis [¹⁸², ¹⁸³]. IFN α/β can induce the IL-10 expression in the T-cells, leading to an immunosuppressive effect. Indeed, the levels of IFN α/β expressed during the infection, the abundance of the signaling and the specific STAT pathway involved can generate different (positive or detrimental) outcomes. [¹⁸⁴, ¹⁸⁵]

IFN α/β exerts a modulator control on NK cells promoting or inhibiting survival and activation. A STAT1 dependent signaling inhibits IFN- γ production by NK cells, while STAT4-dependent signal promotes IFN- γ secretion. [¹⁸⁶, ¹⁸⁷]

IFN α/β plays a dual role also in survival and development of precursor and immature B-cells, promoting activation, antibody response and class switch [188].

The role of Type I IFN is context- and time-dependent. Whereas generally it is important for the resolution of the virus infection, in some cases (in chronicity or in the presence of other factors) Type I IFN leads to disease insurgence, pathogen evasion or symptoms worsening.

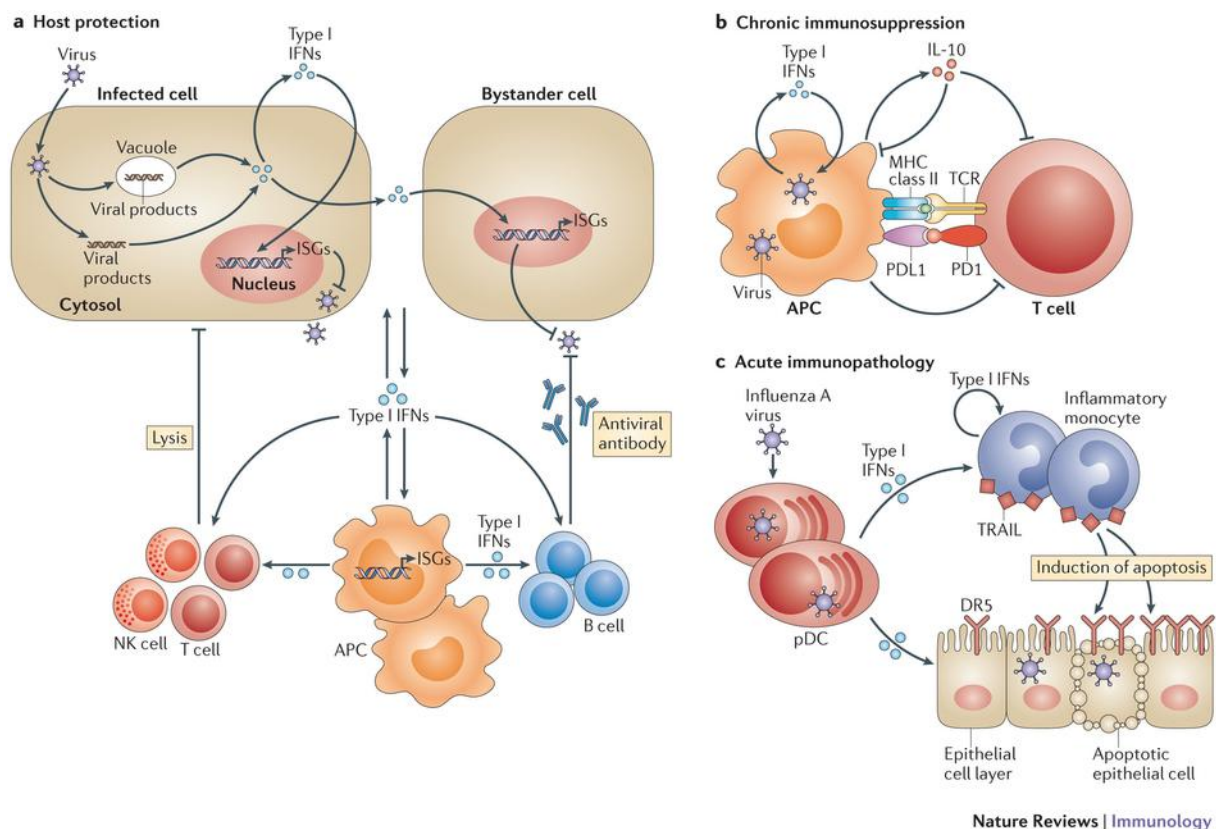


Figure 15: Type I IFN role in host protection and diseases. McNab et al., 2015.

3.2 Role of Type interferon in bacterial infection

As described in the frame of antiviral infections, the type I IFN could play a dual role, namely, by being part of either the solution or the disease.

IFN α/β can induce IFN γ and TH1 immunity, which in turns produces different effector molecules effective against bacteria (as, for example, inducible nitric oxide synthase (iNOS)). Conversely, Type I IFN can inhibit antibacterial factors and pro-inflammatory cytokines, allowing up-regulation of immune suppressive genes. What determines the outcome of a pathogenic or protective response is poorly understood.

Early reports show the protective role of IFN α/β in chlamydial infections as the administration of exogenous IFNs reduces the *Chlamydia trachomatis* intracellular replication in human cells [¹⁸⁹]. IFN α/β induces the expression of indoleamine 2,3-dioxygenase (IDO) depleting the intracellular pool of tryptophan (necessary for the intracellular survival of the bacteria) [¹⁹⁰]. Moreover, the induction of IFN- γ is important for the resolution of the infection. In the *Legionella pneumophila*, the IFN α/β mediates the protection of macrophages and lung epithelial cells (macrophages present a lower load of bacteria and are more polarized to M1). To the same extent, macrophages infected by *Bacillus anthracis* present lower bacterial growth when treated with IFN α/β . [¹⁹¹]

Another effect attributed to IFN α/β is a reduced cellular invasion of the intestinal epithelia cells in *Shigella flexneri* and *Salmonella enterica* infections. Some other evidence suggests a protective role in the *Streptococcus pneumoniae*, *Escherichia coli*, *Helicobacter pylori* and *Streptococcus pyogenes* infections, where *Ifnar1*^{-/-} mice display reduced survival. About the *Streptococcus pneumoniae* infection it has been suggested that IFN α/β contributes to macrophages activation, TNF and IL-6 production. [¹⁹²]

In the *Listeria monocytogenes* infection *Ifnar1*^{-/-} mice are resistant, showing an immune suppressive effect of IFN α/β [¹⁹³]. These mice present an augmented survival rate and a reduction of bacterial load in the spleen and in the liver. Further investigations have revealed that in the presence of IFN α/β there is a large apoptotic cell death, particularly affecting lymphocytes. These cells are sensitized by IFN α/β to listeriolysin O (a toxin produced by *Listeria monocytogenes*), resulting in high level of apoptosis. In *Ifnar1*^{-/-} mice, a reduced lymphocytes apoptosis and a

reduced expression of TRAIL p53 and domain-associated protein 6 (DAP6) were noticed. This massive lymphocytes apoptosis leads to a subsequent production of IL-10, driving a general immune suppression [¹⁹⁴, ¹⁹⁵, ¹⁹⁶]. In *Ifnar1*^{-/-} mice there is also a decrease in the pro-apoptosis genes of the bone marrow-derived macrophages, as IFN α/β leads macrophages apoptosis through a STAT1 mechanism. The myeloid cell apoptosis causes a reduction in protective cytokines production (TNF) and protective iNOS induction. Moreover, macrophages responding to the IFN α/β by down-regulate IFN γ R expression (it has been showed that *Ifngr1* is silenced by transcription repressor). The IFN γ macrophage response is fundamental for eradicating the bacteria, and IFN γ R down-regulation is one of the pathological mechanisms of the *Listeria monocytogenes* immune escape.[¹⁹⁷, ¹⁹⁸, ¹⁹⁹, ²⁰⁰]

In Mycobacteria infections the greater part of the studies highlights the detrimental role of IFN α/β . In 2010 the O'Garra group analyzed the gene expression of large patient cohorts from the United Kingdom and South Africa. They identified an IFN α/β -inducible transcriptional profile in patients with active tuberculosis in the blood; this signature was correlated with radiographic lung disease. It is Interesting to note that this profile was found to diminish with successful anti-mycobacterial treatment [²⁰¹]. Successive studies have verified the presence of this IFN α/β transcriptional profile in additional patient cohorts from Africa and Indonesia. These data suggest a pathological role of Type I IFN in tuberculosis. In the following year, additional evidence emerged from mouse models, where IFN α/β overexpression during the infection was shown to be detrimental [²⁰²]. In hyper-virulent *M. tuberculosis* strains infection, a correlation between increasing levels of IFN α/β and increased virulence was highlighted [²⁰³]. Moreover, Type I IFN administration to the mice's lungs was shown to be deleterious. To the same extent, the administration of substances such (as TLR3 ligand,) capable to induce IFN α/β production, was found to increase the severity of the disease. Another study proved that the knockout of the MAPK kinase kinase 8 (MAP3K8) augmented the bacterial burden in the lung. MAP3K8 is a downstream kinase in the TLR activation acting as a negative regulator of Type I IFN production. Subsequent experiments with double knock-out *Map3k8*^{-/-}*Ifnar1*^{-/-} showed a reduction in the bacterial burden in the lung and diminished levels of IL-10 correlating with increased IL-12 levels in

the serum. Co-infection mouse models with influenza A virus and *M. tuberculosis* displayed disease exacerbation in a Type I IFN-dependent manner. [^{204, 205, 206}]

However, while the detrimental role of IFN α/β in tuberculosis is clear, the mechanism behind it is not yet fully understood. Early studies with hyper-virulent *M. tuberculosis* suggested that IFN α/β production mediates the suppression of pro-inflammatory cytokines and TH1 immunity (both are important for the resolution of the infection). The role of IL-1 α and IL-1 β in the host immunity defense proved crucial, as both are inhibited or reduced by IFN α/β production (confirmed by *in vitro* experiments and in mouse models) [²⁰⁷]. IFN α/β inhibits the inflammasomes which affect the IL-1 β post-translational maturation. Indeed, inhibition of pro-inflammatory cytokines production by the IFN α/β is also extended to TNF and IL-12. Their secretion by DCs and macrophages is negatively affected. On the other hand, IFN α/β induces the secretion of IL-10 and IL-1 receptor antagonist that in turn suppress TNF and IL-12 production [^{208, 209}]. IL-1 α and IL-1 β , on the contrary, can suppress IFN α/β production in mouse models and in human macrophages. IL-1 β leads to the production of prostaglandin E2 that also has an IFN α/β suppressive effect. Prostaglandin E2 administration in mouse infection models can reduce mortality. IL-1 β and prostaglandin E2 contrast the pro-bacterial role of IFN α/β , displaying a complicated balance during the infection [²¹⁰]. As already described, IFN α/β affects the responsiveness of macrophages to IFN γ , another mechanism that leads to immune suppression. A down regulation of IFN γ R expression in the macrophages has been noted in mycobacterial infection. On the other hand, IFN α/β suppresses their ability to produce anti-mycobacterial effector molecules in the macrophages. In mice, Type I IFN induces chemokine productions that attract *M. tuberculosis*-permissive innate cells to the lungs. [^{211, 212, 213}]

Type I IFN has been shown to be detrimental during *Brucella abortus* infection (Ifnar1 $^{-/-}$ mice had a reduced bacterial load, increased IFN γ and nitric oxide production, and less apoptosis), *Yersinia pestis* infection (Ifnar1 $^{-/-}$ mice were more resistant thanks to a higher number of neutrophils with

an enhanced phagocytic function), different GRAM- bacterial infections (where IFN α/β induced an extended cell death).

Interest on microbiota has grown exponentially in the last two decades: its profound impact on the “teaching” of the immune system in early life and its continuous modulation of the immune response have been evinced by a number of fascinating studies [²¹⁴]. In the past two years, three articles helped to define the IFN α/β ’s role in host–microbiota interactions. It has been found that in germ-free mice or in mice under antibiotic treatment IFN-inducible transcriptional response is reduced, with a consequently reduced antiviral immunity. IFNAR knock-out mice showed a proliferation of Paneth cells with altered intestinal microbiota composition. The microbiota induces the production of IFN α/β by the DCs, showed to be protective in a colitis-induced mouse model. These findings show that IFN α/β can play a role in intestinal immune homeostasis. [²¹⁵, ²¹⁶, ²¹⁷]

Viral infections followed by bacterial infections are a significant cause of mortality and morbidity [²¹⁸]. This has proved particularly dangerous when following influenza viral infection; these findings have prompted numerous research teams to seek an explanation to how viral infection sensitizes the host to bacterial infection. In the past few years, IFN α/β has emerged as one of the factors at work in this process. In mouse models, Influenza virus-infected *Ifnar1*^{–/–} mice challenged with a subsequent *Streptococcus pneumonia* infection show increased survival rates compared with wild type [²¹⁹]. A more thorough bacterial clearance has been noticed, whose increased survival rates have been attributed to the increased production of neutrophil and macrophages chemo attractants (such as CXC-chemokine ligand 1 (CXCL1), CXCL2 and CC-chemokine ligand 2 (CCL2)). Despite the rather obscure mechanism underlying the exacerbation of the IFN α/β disease, similarly valuable results can be seen in cases of *Staphylococcus aureus* and *Mycobacterium tuberculosis* infection following a primary viral infection. [²²⁰]

Collectively, these results show that in some bacterial infections (mainly GRAM negative) Type I IFN exerts a beneficial role for the host. As a counterpart, in other infections, especially the ones caused by *S. Aureus*, *L. Monocytogenes* and *M. Tuberculosis*, Type I IFN is strongly linked to the pathogenesis.

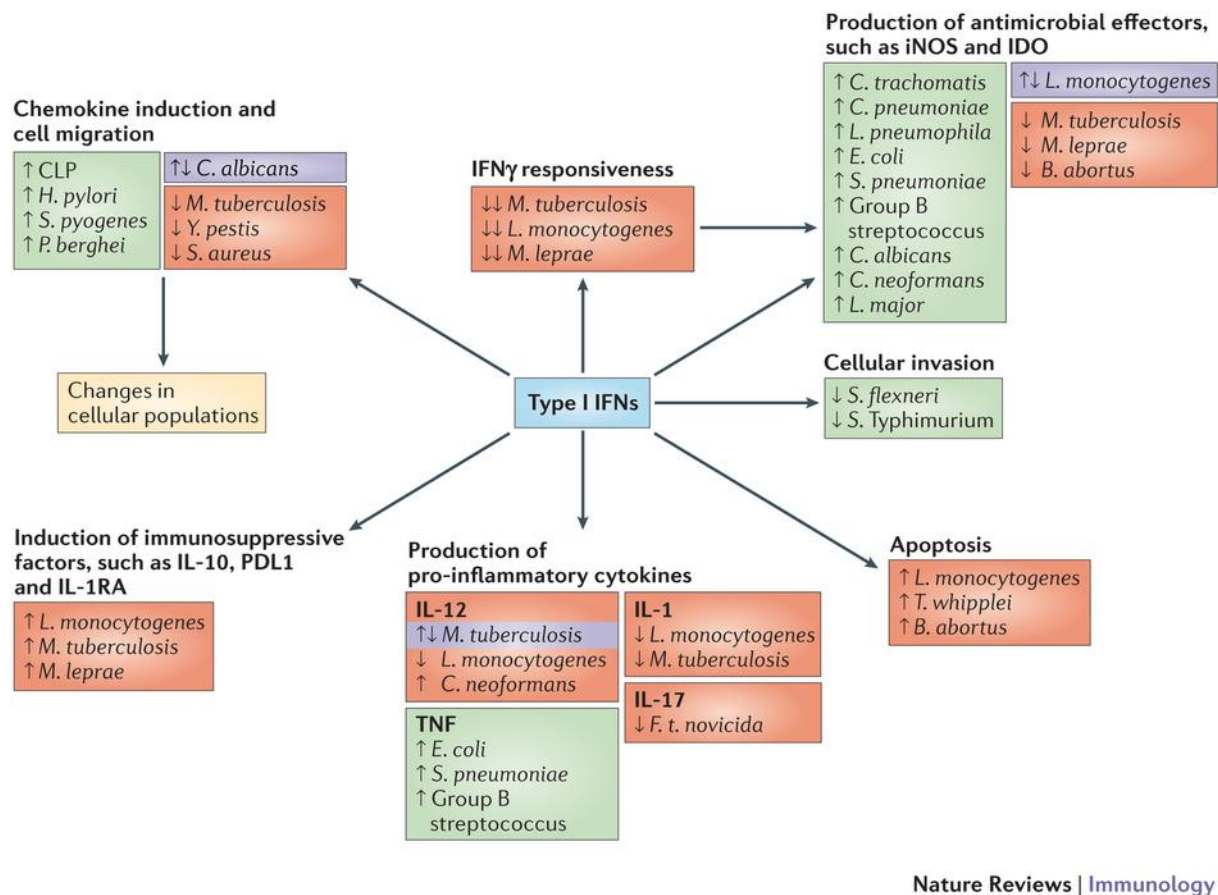


Figure 16: Effects of the Type I IFN on bacterial infections. McNab et al., 2015.

3.3 Type I IFN in parasitic and fungal infections

It is interesting to discuss, however briefly, the role of Type I IFN in parasitic and fungal infections where, once again, its dual role emerges: protective versus pathological. For instance, in *Leishmania*, IFN α/β inhibits the macrophage and neutrophil functions. The role of IFN α/β in

malaria is more complex and depends on both the stage of infection and the *Plasmodium* species. In some cases, such as *Plasmodium berghei* and *Plasmodium chabaudi*, Type I IFN inhibiting the CD4⁺ T-cells functions; in other cases, in *P. berghei* mouse models Type I IFN protects from cerebral malaria disease through a TH1 immunity response. These results may seem conflictual but clearly demonstrate the complexity of IFN α/β impact on the immune response. In the *Trypanosome* infection there are conflicting reports, and IFN α/β plays both a protective and a detrimental role depending on the different factors involved. [²²¹, ²²², ²²³]

In fungal infections, IFN α/β contributes to hosting protective immunity for *Candida albicans*, *Saccharomyces cerevisiae* and *Cryptococcus neoformans*. In these infection models, Type I IFN signaling contributes to the production of reactive oxygen intermediates, to the immune response of TH1 mediated by IFN γ , TNF, iNOS, and to the recruitment of neutrophils. Other studies show its pathological role in *Candida glabrata* and *Histoplasma capsulatum*, though without highlighting its mechanism. Whole exome sequencing and genome-wide association studies found STAT1 mutation in some patients affected by candidiasis and disseminated disease by *H. capsulatum*. STAT1 mutations are gain-of-function and dominants, suggesting the potential role of IFN α/β as detrimental. In conclusion, the role of IFN α/β is still not clear, but it is known to be affected by the conditions and regulation of the system. [²²⁴, ²²⁵]

3.4 Plasmacytoid dendritic cells in bacterial infections

For a long time, pDCs were well known for their pivotal role in antiviral immunity; nevertheless, there were only few reports about their ability to sense and respond to bacteria and fungi. We have already discussed the role of type I IFN in bacterial infections; in this section we shall present more information about direct interactions between pDCs and bacteria.

pDCs are present in the tonsils, where they enter into contact with the bacteria; besides, they are recruited from inflamed tissues (through CCR6). In particular, they are found in bacterial inflamed mucosal epithelia, considered major sites of host–microbe interaction.

Early studies observed Type I IFN induction in *Escherichia Coli* and in *S. aureus* models; however, neither the cells' source nor their mechanism were explained. Subsequent reports showed that pDCs can be a major source of Type I IFN among leucocytes in the peripheral blood. It was further demonstrated that pDCs can uptake *S. aureus*, up-regulate co-stimulatory molecules and secrete cytokines (IL-6, TNF- α , and Type I IFN). The inhibition of the endosomal maturation seems to reduce the production of Type I IFN. pDCs stimulated with *S. aureus* are capable of priming naive CD4+, allowing them to proliferate and polarize in the TH subset. Interestingly, in the tonsils some unidentified soluble factors dampen the production of pDC-derived cytokine as well as Type I IFN. [226]

pDCs can also uptake IgG-bounded *S. aureus* through Fc γ RIIA receptors; this uptake also leads to a Type I IFN release. Due to continuous exposition to *S. aureus*, which is a commensal bacterium, human sera contains IgG specific for *S. aureus*. [227]

Staphylococcal protein A also induces Type I IFN release by pDCs. Albeit this stimulation is weak, protein A is a well-known virulence factor and pDCs Type I IFN release can play a role in the disease. Interestingly, *S. aureus*-activated pDCs stimulate B-cells to proliferate and differentiate. This action is not substituted by exogenous administration of type I IFN but requires cell-to-cell contact. The interaction between pDCs and B-cells leads B-cells to produce IL-10, thus some reports have proposed that *S. aureus* exploit pDCs activation to trigger the polyclonal expansion of regulatory B-cells that suppress anti-bacterial T-cells immunity by the IL-10 production.

Notably, some questions remain open regarding the implication of pDCs in the pathogenic mechanism. For instance, there is a significant IgE production against *S. Aureus* in nasal polyposis, asthma and atopic dermatitis. pDCs could also play a role in Wegener's granulomatosis, where there is *S. aureus* protein A-mediated expansion of B-cells and autoantibody production. [228]

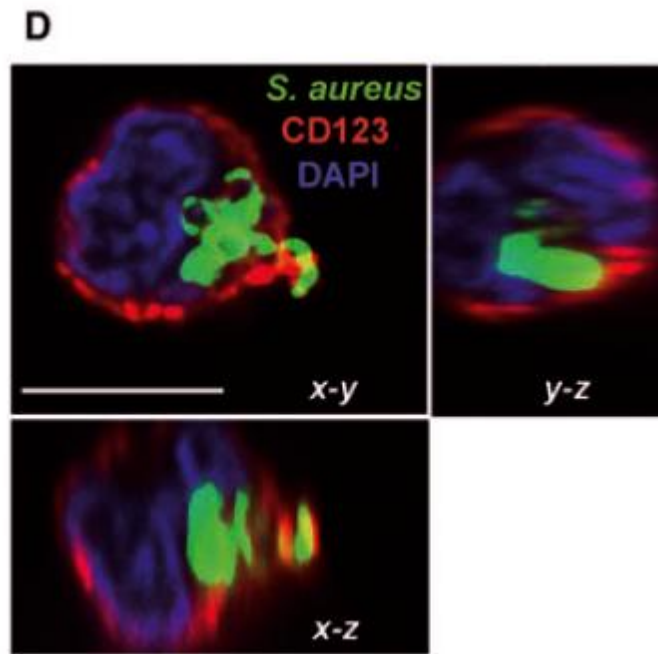


Figure 17: Confocal microscope picture showing that pDCs are engulfing bacteria. From Michea et al., 2013.

Tuberculin skin tests (TSTs) are conducted by intradermal injection of purified protein derivative (PPD) from multiple strains of *Mycobacterium tuberculosis*. A delayed-type hypersensitivity reaction is induced at the injection site on individuals previously sensitized by *M. tuberculosis* or by Bacillus Calmette-Guérin (BCG). PPDs are uptaken by DCs that present the antigen to *M. tuberculosis*-specific memory T-cells, thus inducing a hypersensitivity reaction. pDCs are found in TSTs skin biopsy when hypersensitivity reaction is induced (and not in the control group as pDCs are rarely recruited from non-inflamed tissues). The same team have also found the presence of Type I IFN in the biopsies. Albeit in a lower amount in comparison with mDCs, pDCs are also capable to uptake PPDs. [²²⁹]

pDCs are normally present in the lymph nodes in the T-cell zone but they are located in the B-cell area. In tuberculosis (TB) patients LNs, pDCs were found in a large number, clearly distinguishable,

and in contact with CD3+ (T-cells) or CD20+ (B-Cells) cells. pDCs have proved able to produce Granzyme B in response to *M. tuberculosis* or BCG stimulation. In the TB patients LN pDCs were found to contain Granzyme B. [²³⁰]

It is known that pDCs can sense GRAM+ bacteria, but it is much less clear how they do it and their role in the bacterial infection. However, as described in the previous paragraph, the viral infections followed by the subsequent bacterial infection are fatal, in mouse model. The recruited pDCs secrete high levels of type I IFN during the immune response against virus. The results presented in the previous chapter and the previously mentioned deleterious role of Type I IFN, give us an indication of the potential detrimental pDCs impact during a GRAM+ infection.

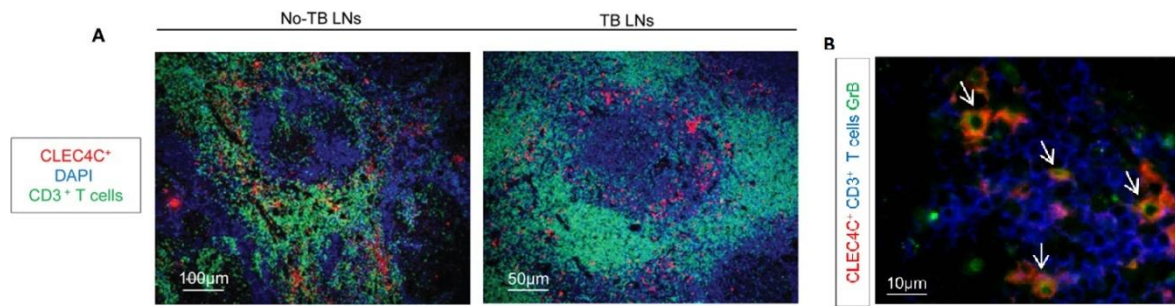


Figure 18: increased presence of pDCs in TB patient LN **A.** pDCs (CLEC4C+ cells) increase in the TB patients LN T-cell zone. **B.** pDCs (CLEC4C+ cells) Granzyme B (GrB) in the TB patients LN T-cell zone. Adapted from Lozza et al., 2014.

4. Choose your fate *a priori*

4.1 Receptor regulation complexity

The Lorenz laws in biology

Does the flap of a butterfly's wings in Brazil set off a tornado in Texas?

Edward Lorenz, 1979

In 1960 Edward Lorenz was working on a weather forecast modeling. The complex mathematical calculations this involved led him to try small approximations in the number of digits. Surprisingly, he found that even the smallest approximation led to outstanding change in the final model. The so-called “butterfly effect”, named after the title of his famous 1972 talk, has now become a much-beloved metaphor. In the same vein, on proposing the chaos theory, Lorenz had written *“One meteorologist remarked that if the theory were correct, one flap of a sea gull's wings would be enough to alter the course of the weather forever. The controversy has not yet been settled, but the most recent evidence seems to favor the sea gulls.”*

Effectively, chaos is the reason why we cannot forecast a precise weather model. The weather presents a chaotic dynamic, based on three properties:

1. it must be sensitive to initial conditions
2. it must be topologically mixing
3. it periodic orbits must be dense

One fundamental paradigm of biology is homeostasis, i.e., the property of a system in which a variable is actively regulated to remain very nearly constant. Examples of homeostasis are: the body temperature regulation, the pH in extracellular fluids, the ions' concentration (Na^+ and Ca^+), the partial pressure of oxygen in blood, carbon dioxide partial pressure, arterial blood pressure, the volume of body water, and the glucose in the blood plasma. The metabolism of each living

being spends a considerable amount of energy to maintain these variables stable during changes in the external environment. Dysregulation of homeostasis mechanisms may lead to death or the insurgence of a severe disease state, such as an excessive increase in the body temperature or an excessively high glucose plasma concentration (in diabetic subjects). Variations in the variables are measured by different sensors leading to the activation of a correcting system to recover stability. In mammals, for example, the body temperature is tightly regulated, and when hypothalamic temperature sensors notify a decrease in the temperature blood flow is regulated to preserve body heat; conversely, sweating is induced to cool down the body through water evaporation. It is easy to understand the need for homeostatic behavior, less so the advantages of chaotic dynamics. One example is the neuronal integration of the signal, where a small change in stimulus integration leads to the activation of different brain neurons. Such a behavior allows a sophisticated response to minimal changes in the environment. The nonlinear dynamics of a chaotic system facilitates the extraordinary ability of neural systems to adapt, making transitions from one pattern of behavior to another when the environment is changed and, consequently, creating a rich variety of patterns. [²³¹]

TLRs activation can be considered a chaotic system. It depends from the initial conditions (amount of receptors present on the surface or in the endosome, presence of different adaptor proteins, co-receptor expressions, negative o positive regulators, epigenetic state of the cells, integration of a number of other signals). An alteration in these initial conditions can lead to a different outcome. Topological mixing (or topological transitivity) means that the system will evolve over time, so that any given region or open set will eventually be influenced. TLRs activation induces a strong response in the cell, leading to an exponential amplification of the signals thanks to recruitment of different molecules. Likewise, Type I IFN immune effects follow a chaotic dynamic and, more broadly, we can assume dendritic cells to behave in a similar fashion. DCs cytokines production and co-stimulatory expression patterns decide the T-cells' polarization fate. How the DCs choose these different patterns is the result of the signaling integration coming from the environment. Changes in the initial stimuli lead to profoundly different outcomes. Another example is the TGF- β in tumorigenesis, either acting as a tumor suppressor or facilitating the

tumor's progression according to the initial conditions.^[232] The engagement of TLR receptors and the role of Type I IFN in bacterial infections have shown conflictual results, leading the scientific community to unsatisfying forecasts. These results can be explained by a chaotic dynamic, which means it is important to further investigate the hidden factors that allow this behavior.

Complexity and plasticity

Pathogens use largely complicated, diverse and efficient strategies to escape the immune system which confronts the new challenges through adaptive immunity. *Plasmodium falciparum*, for instance, undergoes an antigen variance. The plasmodium genome codifies for 60 VAR genes, but only one of these genes is expressed each time and is present on the surface of infected red blood cells. The other 59 genes are epigenetically repressed during this phase. While the immune system generates a response against this antigen, the parasites shut down the expression and express another gene, thus escaping the infection's resolution ^[233].

In the *Candida albicans* infection two forms of fungi can be present: unicellular yeast and hyphae. These morphological transitions are reversible and this plasticity is believed to facilitate pathogenicity. While *C. albicans* as unicellular yeast is generally considered a case of commensalism (a harmless colonization), the hyphal growth is associated with mucosal infections. Unicellular yeast can disseminate throughout the body. The balance between commensalism and infection relies on the immune system; the pathogenic potential is determined by the effectiveness of the host's immune response. Dendritic cells that phagocyte *C. albicans* yeast cells secrete IL-12, which is functional to the induction of TH1 T-cells. TH1 cells stimulate macrophages to kill the phagocytated pathogens. Macrophages are not capable to phagocyte hyphae, thus the TH1 response is not protective. TH17 cells, instead, are effective against hyphae forms. Moreover, TH17 are important in the resolution of the mucosal infections where *C. albicans* hyphae is present. The immune system has to decipher the threat in order to use its most effective weapons. ^[234]

Classically, TH subsets are defined by the cytokines they produce.. The first subsets identified were TH1 and TH2. TH1 cells produce IFN- γ and are important in immunity to viruses, intracellular pathogens and cancer. Th2 cells secrete IL-4, IL-5 and IL-13 and promote immunity to large extracellular pathogens such as helminthes. During the last few years other subsets have been identified such as Th17 (cells producing IL-17, which induces the release of antimicrobial peptides on mucosal surfaces and plays a prominent role in antifungal immunity), Tfh cells (which secrete IL-4 and IL-21, crucial to regulating B cell responses). T-cells can be differentiated as T-regs ,secreting IL-10 and playing a role in the regulation of the immune response to avoid over-inflammation and tissue damage. T-regs can also prevent autoimmunity and avoid an immune response against self-antigens. In addition, the newest characterized subsets are: IL-9 producing Th9 cells implicated in worm expulsion, and IL-22-producing Th22 cells helpful in wound healing. It is not excluded that in the next few years other subsets will be added to this list (some have already been proposed). A number of nuclear proteins, known as *master regulators*, play a pivotal role in T-helper cell subset differentiation The best characterized are T-bet, GATA3, ROR γ t, Bcl-6 and Foxp3 , critical to, respectively, Th1, Th2, Th17, Tfh and iTreg cell differentiation. In the GRAM+ infection, the TH1 mediated response is generally considered protective. For instance, in Mycobacterial infections, TH1 produced IFN- γ enhances macrophage microbicidal mechanisms through iNOS pathway induction and stimulates phagosomes maturation and autophagy. TH2 IL-4 production in experimental models shows an association of increased IL-4 with progression of tuberculosis and reactivation of the disease. In humans, some studies have demonstrated that the increased levels of IL-4 in the blood and in the bronchoalveolar lavage fluid (BALF) of TB patients are correlated to the most severe form of the disease. Moreover, IL-4 levels decrease with the progression of the treatment, while IL-4 and IL-13 inhibit the autophagy process and impact MTB antigen presentation, T-cell clonal expansion and the organization of granulomas [²³⁵, ²³⁶, ²³⁷]. Interestingly, in MTB infection T-regs play a role in preventing TH mediated immunity from inducing a more extended tissue injury than the pathogen mediated one, with a particular importance in the early phase of the infection. T-regs are also responsible for reactivating the silent pathogen: by being present in higher levels in individuals with a more severe active disease, higher levels of Tregs delay response to the treatment, thereby reducing IFN- γ overall levels [²³⁸] .

TH17 are dispensable in TB immunity. Interestingly, in cultures of PBMCs in patients with active TB there is an intense production of IL-22 and IFN- γ by distinct subsets of CD4⁺ T cells; patients with tuberculous pleural effusion have an increased concentration of IL-22 and Th22 cells in their pleural fluid samples. Concretely the role of this subset in TB is unknown. In the last few years, the presence of TH9 cells has been shown in TB in pleural effusion and, compared to evidence from health controls, patients with pulmonary TB had significantly higher levels of IL-6 and IL-9, with this latter antagonizing IFN- γ , suggesting that it can play a role in the pathology. However, while TH1 play a role in autoimmune disease, TH17 are involved in psoriasis and other pathologies. TH2 and TH9 are involved in allergy and asthma. T-regs in tumors play a role in blocking the immune-mediated response. The plasticity and complexity of these subsets produce precise responses to individual threats, but small alterations in this delicately regulated balance invite disease. [²³⁹, ²⁴⁰, ²⁴¹, ²⁴², ²⁴³]

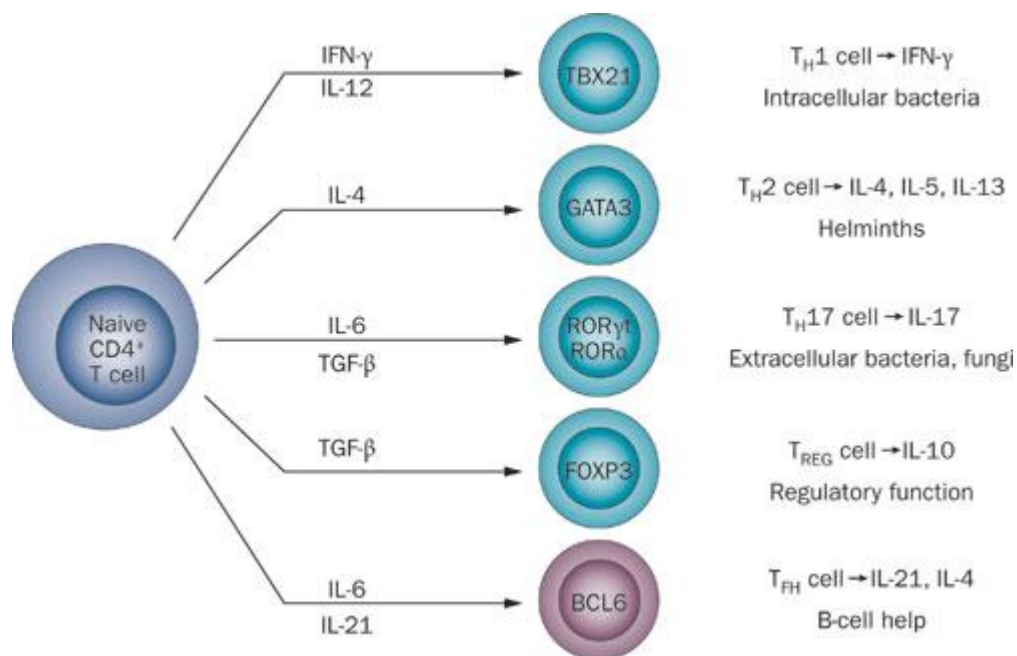


Figure 19: TH subsets and their role. From Craft et al., 2012.

In the previous chapters the role of DCs was described along with their capacity to present antigens to up-regulate co-stimulatory molecules and secrete cytokines. pDCs have all these capacities; they also show unique features in the DCs family, such as the production of Granzyme B and TRAIL-mediated killing. Besides, pDCs show other remarkable abilities and different behaviors from what can be expected from DCs. For instance, in *Aspergillus fumigatus* infections, the most common cause of invasive mold infections, an unusual pDCs role has been found. The large size of hyphal morphotype prevents phagocytosis, while neutrophils with the formation of neutrophil extracellular traps (NETs) show direct antifungal activity. In the presence of *Aspergillus fumigatus hyphae*, pDCs produce IFN- α and TNF- α , but are also capable of direct killing [²⁴⁴]. pDCs express zinc- and iron-binding proteins, such as calprotectin and lactoferrin (generally expressed by phagocytic populations), leading to chelate the divalent cations essential for fungal growth. The interaction between pDCs and *A. fumigatus* induces the formation of pDCs extracellular traps (pETs) containing DNA and citrullinated histone H3 [²⁴⁵]. These structures closely resemble those of neutrophil extracellular traps. This mechanism is known as ETosis (detected in neutrophils and rarely reported in other cell types). The dying cells generate these extracellular traps composed of chromatin assembled with antimicrobial proteins capable to trap and kill pathogens (generally fungi and bacteria). This active process requires chromatin decondensation mediated by histone citrullination, and only a small percentage of cells undergoes this process. This mechanism is important for contributing to the extracellular anti-microbial host defense. This demonstrates the plasticity of pDCs, capable to adapt differently to the pathogen. [²⁴⁶, ²⁴⁷]

While the T-helper plasticity and the need of a tuned adaptive response for the infection resolution are commonly accepted, the latter is more noticeable. pDCs can acquire different capacities in unexpected contexts, which encompass their traditional role of virus sentinels. It has been showed that PET formation is activated by Dectin-2, an un-characterized receptor on pDCs. The activation of different receptors can potentially lead to new features.

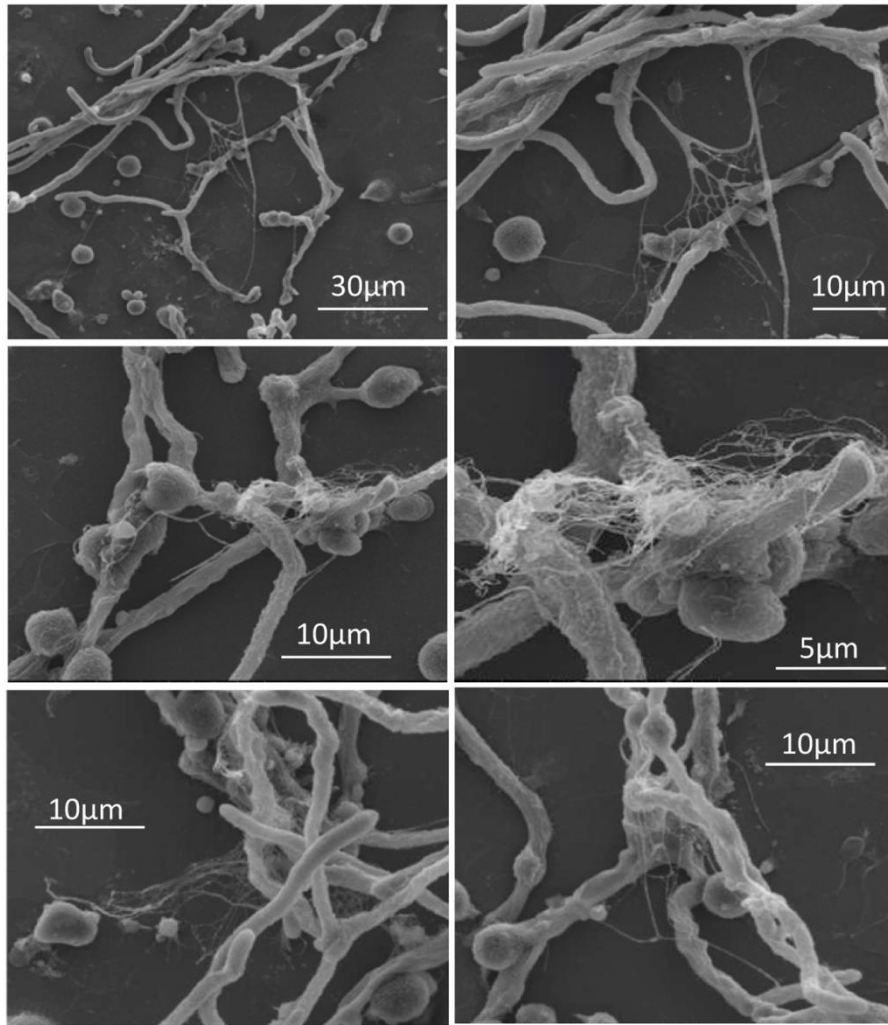


Figure 20: Scanning electron microscopy pictures of pDCs stimulated with *Aspergillus fumigatus* hyphae. Areas with pDCs ETs are shown at low (left panels) and high (right panels) magnification. From Loures et al., 2015.

Signal integration

During inflammation, dendritic cells receive numerous signals that they have to identify which threat they need to face. The different patterns of receptors engaged decide the ultimate response as these signals are integrated in specific outputs. As described in the previous chapter, Type I IFN production of pDCs can be modulated from the engagement of other receptors on the cell's surface. An example of positive regulation: *Porphyromonas gingivalis* induces the formation

of the biologically active C5a (one component of the complement cascade); this leads to an intracellular Ca^{2+} signaling which synergistically enhances the TLR2 mediated in macrophages. These systems lead to fine-tuning for generating a more appropriate response to the microbe. [248]

Another interesting feature of TLRs signaling is the presence of different regulators. TLR4 stimulation induces IRF4 expression; IRF4 interferes with the MyD88/IRF5 complex, dampening its response. IRF4 competes with IRF5 to bind MyD88, reducing the activation. AFT3 is another negative regulator that remodels the chromatin structure to suppress IL-6 and IL-12 p40 expression. If AFT3 is induced at an early time point in TLR4 activation there is a suppression of IL-6 and IL-12 p40 production. SOCS-1 is a potent regulator, its activation blunting TLR2 and TLR4 signaling. Interestingly, there are other negative regulator proteins that suppress only specific branches of TLRs activation. Dok1 and Dok2 do not affect NF- κ B activation but inhibit the MAP kinases cascade. β -arrestins, instead, interfere with the TRAF6, blocking NF- κ B activation. The signaling is regulated in all its components, p50 proteins are induced by LPS stimulation and their expression is increased during successive exposures to the endotoxin. p50 homodimers bind NF- κ B sites in the nucleus but lack the transactivation domains, thus preventing NF- κ B binding. [249, 250, 251, 252, 253, 254]

The dendritic cells integrate these PAMPs signals with cytokines, DAMPs and other inputs. The effect of this combination of signals cannot be predicted from the effect of input alone. In general, the result would involve either of two scenarios : synergism and antagonism. Synergy occurs when the collective response of the signals is higher than the product of the two separate signals. Antagonism is the contrary.

In macrophages, pre-incubation with LPS (TLR4 Ligand) increases nitric oxide induction in response to CpG DNA (TLR9 ligand), but the reverse reduces this production. This and additional data show that synergism is time dependent. Literature reports different synergisms in TLRs activation, as LPS and bacterial and lipoproteins (TLR2 ligand) induce pro-inflammatory cytokines. In addition, as TLRs stimulation changes the mRNA TLRs expressions, an up-regulation of TLRs mRNA has been shown in monocytes and dendritic cells after their activation. Likewise, TLRs agonists induce up-

regulation in the mediators' expression (as described before for IRF7). TLR4 mRNA has been reported to be down-regulated in response to LPS in some cases. [²⁵⁵, ²⁵⁶, ²⁵⁷,]

As described in the first chapter, there are different crosstalk levels among the immune cells, as CpG DNA NK stimulation leads them to produce IFN- γ that acts on the macrophages to augment their responsiveness to LPS. In general, cytokines stimulation affects positively and negatively the cells' response to TLRs agonists. This happens at various levels:

- Regulation of the TLRs receptors, co-receptors and adaptor proteins expression.
- Crosstalk with the negative regulators of TLRs signaling.
- Positive or negative regulation of the target gene promoter.

The administration of IFN- γ to macrophages induces the expression of TLR2, 4, 6 and 9 and co-receptor CD14, and MyD88 and IRAK1 (process called APC priming). In response to LPS, primed monocytes show an increased production of TNF- α , due to higher mRNA stabilization. Similar effects are reported for IL-4, Type I IFN, GM-CSF and M-CSF. Prolonged cytokine exposition up-regulates TLR negative regulators such as SOCS-1. IL-10, TGF- β , arachidonic metabolites and glucocorticoids dampen TLR response. The glucocorticoid receptor engagement interferes with NF- κ B activation. Positive and negative loops, as well as spatial and temporal integration, can modulate this response by introducing a further level of complexity. [²⁵⁸, ²⁵⁹, ²⁶⁰, ²⁶¹, ²⁶², ²⁶³]

Synergies and antagonisms help to regulate and adjust the overall response; however, this view is largely reductive and multiple scenarios can be detected. This complexity has been observed, for instance, in how pDCs integrates IL-3 and influenza virus stimulations, showing unexpected emerging features. [²⁶⁴]

4.2 Toll-like Receptors dysregulation

TLR expression alteration in bacterial infection

There is a wide range of variability in infectious disease outcomes among different individuals and advancement in genotyping techniques shows genetic factors of the host influencing susceptibility to this disease. The outcome is also dependent on cytokines production in response to the infection, which has been shown to be influenced by genetic factors. Interestingly, in some human diseases a single gene defect can increase susceptibility. The conflictual interaction between TLRs and bacteria has been already described. Here the focus will be on how alterations in their expression impact the immune response. TLRs receptor genes defects have generally been linked to diseases and to insufficient response to pathogens. In the genome, one common variation is the single nucleotide polymorphism (SNP). Some of these SNPs alter expression of TLRs or their function. [²⁶⁵, ²⁶⁶]

A non-synonymous SNP, Arg753Gln (G2258A) (rs5743708), in the TIR domain of TLR2 was identified in the year 2000. This SNP is a missense mutation leading an arginine to glutamine substitution. This mutation leads to a decrease in the activation. A reduction in TLR2/TLR1 signaling and protection against the development of late-stage Lyme disease has been shown. This mutation was found to reduce the response against *Staphylococcus aureus*, and was found associated with recurrent bacterial infections in Turkish children. People carrying this SNP were found to be more susceptible to urinary tract infection with Gram-positive pathogens, presenting more frequent infections. In patients with *Herpes simplex* viral infection, mutation in TLR2 seems to play a role in augmenting viral shedding and lesional rate. Moreover, TLR2 SNPs are linked in post transplantation infections, allograft failure and mortality. Different polymorphisms of TLR2 are associated with Tuberculosis. The synonymous SNP 597CC is reported to be strongly associated with meningeal TB and miliary TB (disseminated forms of Tuberculosis outside the lung) in Vietnamese patients. [²⁶⁷] Some TLR2 SNPs are linked to different cancers: in lymphoma progression, and related to higher risk of gallbladder cancer, breast cancer, and gastric cancer in the Brazilian population, bladder cancer in the North Indian population, and cervical cancer in North Indian women. Individuals with SNP 597CC show a fivefold decreased risk of colorectal

carcinoma. TLR2 SNPs are associated with rheumatic fever, preterm birth and pancolitis. [²⁶⁸, ²⁶⁹, ²⁷⁰, ²⁷¹]

In a population-based study in Sweden, eleven TLR1 SNPs were reported: among them, three were found to be associated with an increased risk of prostate cancer. Three SNPs were found in the central extracellular region (Asn248Ser (A743G), H305L (A914T), and P315L). These SNPs lead to diminished sensing of microbial cell wall components *in vitro*. Interestingly, one of these SNPs (P315L) is part of an epitope for an inhibitory anti-TLR1 monoclonal antibody. After stem cell transplantation, the presence of the SNPs Arg80Thr and Asn248Ser in the recipients make them more susceptible to aspergillosis. The most common is SNP is S602I, is localized at the junction of the transmembrane and cytoplasmic domain of the receptor. This SNP shows a tenfold decrease in IL-6 production by peripheral blood leukocytes. This SNP alters the trafficking of TLR1 to the cell surface; the receptor is retained in the Golgi apparatus. This SNP was found to be protective against leprosy in a Turkish cohort. [²⁷², ²⁷³]. Moreover, it provides protection against *Helicobacter pylori*-induced gastric diseases. This SNP is also connected with the reason why the *Borrelia* lipoprotein vaccine trial failed. It also has a significant association with mild malaria, ileal disease, and TB in Africans, Americans and Hispanics. [²⁷⁴, ²⁷⁵, ²⁷⁶, ²⁷⁷, ²⁷⁸]

TLR role in autoimmunity

Toll-like Receptors recognize foreign structures. Mammals do not produce LPS or flagellin, and endosomal confinement should prevent their recognition of endogenous mRNA or DNA. While it is clearer that some dysregulation can lead to autoimmunity in the endosomal TLR case, the discovery that surface TLRs can bind endogenous DAMP offer new suggestions. It has been reported that synovial fluid macrophages in the arthritic joint recognize PAMPs and DAMPs from necrotic cells. In rheumatoid arthritis, there is a high content of low molecular weight hyaluronic acid and RNA release that can be recognized by TLRs. There is a high infiltration of neutrophil polymorphs, macrophages and lymphocytes and these are all cells that can be activated by TLRs binding. It is known that the pro-inflammatory cytokines in the joint lead the macrophages to differentiate into bone resorbing osteoclasts. TLR9 plays an anti-inflammatory role in arthritis

through the induction of tolerance. TLR9 Agonists decrease severity in mouse model. TLR2 can recognize HMGB-1 and lead to the production of pro-inflammatory cytokines [²⁷⁹]

Type I diabetes (T1D) is an autoimmune disorder in which the pancreatic β cells (the cells present in the islet of Langerhans which secrete insulin) are selectively destroyed by T-helper 1 (TH1) cells. Its etiology is still far from being elucidated but a combination of environmental and genetic factors contribute to its pathogenesis. TLRs play a role in the development of T1D [²⁸⁰]. Mice treated with TLR3 agonist and insulin have developed insulinitis; furthermore, TLR7 engagement leads to autoimmunity [²⁸¹]; pancreatic β cells up-regulate MHC-I molecules. TLR2 activation of DCs contributes to the onset of T1D in non-obese diabetic mice by inducing β -cell death. Mice knockout for MyD88 do not develop diabetes. Other studies show up-regulation of TLR2, TLR6 mRNA in PBMCs with respect to healthy controls; moreover, TLR2 and TLR4 ligands in T1D patients are significantly increased. TLR2 SNPs are associated with T1D in Norwegian and Korean populations. TLR1 SNPs associations with T1D patients were found in a large Chinese cohort. [²⁸²,
²⁸³, ²⁸⁴]

Systemic lupus erythematosus (SLE) is a systemic autoimmune condition characterized by B cell hyperactivity and the presence of various circulating autoantibodies, often directed to dsDNA and RNA-binding proteins. Its etiology is multifactorial: genetic, environmental factors, UV light, drugs, infections and immune system alterations have been proposed as causes. SLE patients show a marked increase in the apoptosis with a consequent release of DNA and RNA that can be sensed by TLR9 and TLR7. A diminished susceptibility to the onset of SLE has been reported in the presence of a SNP affecting MAL involved in TLR4 and TLR2 signaling pathways. [²⁸⁵]

Systemic sclerosis (SSc) is a connective tissue pathology with initial vascular injuries followed by exaggerated extracellular matrix (ECM) and collagen production, due to fibroblasts hyperactivation. The fibrosis in the mild forms are limited to the skin with some vascular complications; in the most aggressive cases, they extend to the internal organs, progressively leading to their collapse. Several studies support the role of TLRs in its pathogenesis. Among the endogenous TLRs ligands there are proteins, matrix-derived molecules, immune complexes and

nucleic acids. SNPs in TLR4 in mice showed a reduction in a skin sclerosis-induced model. HMGB-1 is present in serum and lesional skin and, through the up-regulation of transforming growth factor β (TGF- β) signaling, it induces fibroblast activation. SNPs have been found in TLR2 associated with the pathology (antitopoisomerase positivity), as well as higher levels of pro-inflammatory cytokines. [²⁸⁶, ²⁸⁷]

Crohn's disease (CD) and ulcerative colitis (UC) represent the two principal forms of idiopathic inflammatory bowel disease (IBD). IBD patients present a chronic inflammation affecting the gastrointestinal tract and frequent extra-intestinal manifestations. It has been suggested to be dependent by dysregulation of the innate and adaptive immune responses against PAMPs to the intestinal flora as part of the pathogenesis. TLR4 SNPs are associated with IBD and confirmed by enhanced expression in epithelial cells, I macrophages and DCs infiltrating in the inflamed mucosa. TLR1, TLR2, and TLR10 SNPs are associated with IBD (these receptors can all heterodimerize). Two independent study confirmed TLR10s' association with IBD. [²⁸⁸, ²⁸⁹, ²⁹⁰]

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), characterized by immune mediated demyelination and damage of axons, and whose complex etiology is not fully disclosed. TLR2 and TLR4 expression levels in PBMCs were found significantly higher for both in MS patients compared to healthy controls. [²⁹¹]

Different TLR2 and TLR4 SNPs were found associated with vitiligo. Myasthenia gravis (MG) is characterized by auto-antibodies recognizing several components of the neuromuscular junction (NMJ) and causing muscle weakness and early fatigability. One of the hypotheses about its insurgence is that the infections represent triggering factors for MG (in concrete, microbial antigens could promote the activation of T-cells against self-antigens similar to the foreign molecules). The PBMCs MG patients show TLRs expression alterations compared with healthy controls: TLR2, TLR3, TLR4, TLR5, TLR8 and TLR9 expression levels were enhanced, whereas TLR1, TLR6 and TLR10 were diminished. [²⁹²]

TLR2, TLR1 are implicated in different autoimmune diseases, where pDCs and type I IFN are also involved. Deregulation of TLR7 and TLR9 expression in pDCs has a role in the insurgence of SLE and

other pathologies. TLR2, TLR1 and TLR6 expression in pDCs is still being debated, but it may be interesting as a future perspective.

OBJECTIVE OF THE THESIS

This thesis project originates from a different initial question "what is more important in the priming of T-cells immunity: the DC's subset or the stimuli?"

Two models have been proposed:

- *Evolutionary theory*: differences in the DC subset decide T-cell priming and the same stimulus administration to different DC subsets leads to different T-cell profiles.
- *Environmental instruction theory*: the stimulus is the main determinant in T-cell commitment and the same stimulus administration to different DC subset leads to an equal T-cell profile.

To assess which model was predominant, I treated different DC subsets with a number of stimuli that can be recognized by those cells.

As stated in the history of science, a rigorous and systematic approach has been fundamental for achieving the results; however, serendipity has also played an important role. During these experiments, I found that pDCs were capable to respond to TLR2/1 ligands. This unexpected discovery opened new, interesting questions.

In the introduction, I presented the essential features of pDCs and how they are connected with other cells, showing that they are a node in a complex network. In the second chapter, I described GRAM + bacterial infections and how they escape immune sensing. Moreover, I focused on the role of Type I Interferon, showing its dual nature in the microbial infection. pDCs are the cells capable to secrete the highest amount of Type I Interferon, posing the question of whether or not they are involved in bacterial infection. Despite their role being still controversial, evidence from the last few years reported in the second chapter of this manuscript suggests that pDCs can respond to the GRAM+ bacterial infection. In the third chapter, I presented the receptors involved in bacterial sensing and focused on TLRs, particularly TLR2/1. Different questions have come up because of the important involvement in the sensing and the immune escape of GRAM+ bacteria:

1. Do pDCs express TLR2 and TLR1?
2. Are TLR2 and TLR1 functional in pDCs? Do pDCs respond to Lipoproteins?

3. Are TLR2/1 involved in pDC bacterial sensing?
4. Are pDCs capable of inducing T-cell polarization?

Since the results of this thesis gave positive answers to these questions, I was interested in further researching the role of these receptors in pDCs. In the fourth chapter I demonstrated how a small dysregulation of this receptors' expression undergoes a dramatic change in the outcome. The imbalance of these receptors is deeply involved in different pathologies, causing reactions that go from failure to provide a protective response to the microbial infection to the capacity of developing autoimmunity. Likewise, the pDCs' abnormal response has an impact on autoimmune diseases. An in-depth characterization of the TLRs' response on pDCs will allow us to better understand the role of pDCs in the immune system. So far, a strong effort has been made to understand TLR7 and TLR9 in pDCs, as it was thought they were the only TLRs expressed by them. The additional questions I addressed were:

1. Do TLR1 and TLR2 exert the same function on the pDCs' lipoprotein activation?
2. Are these difference reflected on T-cell priming?
3. Do these differences rely on a different intracellular signaling?

The answers to all these questions are that pDCs express functional TLR2 and TLR1, which allows them to fully respond to bacterial lipoproteins. This pathway is involved in their capacity to sense Gram+ bacteria. When stimulated with GRAM+ bacteria, pDCs are capable to induce a T-cell response.

Surprisingly, in the pDCs' response to lipoproteins TLR1 controls co-stimulatory and pro-inflammatory cytokines production, T-cells proliferation and expansion, and TH2 and Tregs priming. TLR2, instead, controls Type I IFN production. These differences rely on different signalization.

RESULTS

6. Submitted article

TLR2/1 orchestrate human plasmacytoid dendritic cells response to Gram+ bacteria

Authors: Salvatore Raieli, Vassili Soumelis

While Tuberculosis and multi-drug resistant bacteria are becoming a serious threat for our health, we are still far from a comprehensive knowledge of the host microbe relationship. Whilst we are still surrounded by open questions, there are numerous reports establishing the pathogenic role of Type I Interferon in the skewing from a protective to a failing immune response.

Plasmacytoid dendritic cells (pDCs) are a subset of dendritic cells capable to respond by secreting high amounts of Type I interferon when challenged with TLRs ligand or during viral infections. pDCs are able to respond to GRAM+ bacteria and there is further evidence of their potential implication in the disease; nevertheless, their role in the bacterial infection needs to be investigated further.

In the current view, pDCs sense the nucleic acid of GRAM+ bacteria through the endosomal TLRs. This assumption clashes with the poor phagocytic activity of the pDCs that limits their capacity to engulf the bacteria and deliver them to endosomal TLRs. Moreover, TLR response to GRAM+ bacteria is considered to be digital without shades, but this view collides with the conflictual results obtained by knock-down experiments and *in vivo* infection models.

In this study, we show the presence of TLR2 and TLR1 on a pDC surface that allows them to respond to GRAM+ bacteria without need of internalization. pDCs functionally respond to bacterial lipoprotein (natural ligand of these two TLRs) and we noticed that the two receptors have no redundant functions. TLR1 is more devoted to controlling surface molecules and the cytokines implicated in T-cell polarization; conversely, TLR2 exert control on the Type I IFN which acts on a broader stage. Our results explain the mechanistic reason behind these behaviors, showing the engagement of different pathways from the two TLRs.

These results help answer the question of how pDCs sense the GRAM+ bacteria. Our work allows to further characterize TLR2 and TLR1 behavior and their implications. We have introduced a new level of complexity in the system by challenging the current view that when two receptors cooperate they transmit the same signal. We have furnished a springboard to new intriguing questions leading to a better characterization in the frame of bacterial infection and provide further insights in autoimmune diseases.

Title: TLR2/1 orchestrate human plasmacytoid dendritic cell response to Gram+ bacteria

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Abstract:

Tuberculosis is worldwide life-threatening disease. Moreover, infections by Gram+, multi-drug resistant bacteria are emerging as a cause of mortality. In both cases new studies have highlighted the pathological role of Type I interferon (IFN). Plasmacytoid dendritic cells (pDCs) produce high amounts of Type I IFN following viral sensing. Recent evidence suggests that human pDCs might sense bacteria. However, human pDCs are reported to express only endosomal pattern recognition receptors, Toll-like receptors (TLR)-7 and TLR9, mostly associated to viral sensing. The receptors mediating bacterial sensing in pDCs are not known.

We show here that human primary pDCs express TLR1 and TLR2 at the mRNA and protein level. We demonstrate that pDCs respond to TLR2/1 synthetic ligand (PAM3CSK4), as well as Gram+ bacteria *Mycobacterium tuberculosis*, *Staphylococcus aureus* and *Listeria monocytogenes*, with the up-regulation of costimulatory molecules (as MHCII, CD80 and CD86) and cytokine secretion (IL-6, TNF- α , IFN-I). pDCs activation in these settings is blocked by a TLR2/1 competitive antagonist (CU-CPT22). In human primary pDC we have found that in response to bacterial lipoproteins up-regulation of costimulatory molecules is TLR1-dependent while IFN secretion is TLR2-dependent. TLR2 and TLR1 play a different role in pDC priming of naïve CD4+ T-cells, inducing proliferation and T-helper cell differentiation. We further demonstrate that these differences rely on the diverse signaling pathways activated by the two TLRs (PI3K, MAPK, NF- β).

Overall, we show that pDCs sense Gram+ bacteria through the TLR2/1 pathway, and suggest a differential role for TLR1 and TLR2 in the induction of type I IFN versus T-cell activation. This work provides the rationale to explore and target pDCs in human bacterial infection.

Introduction

Tuberculosis (TB) and multi-drug resistant bacteria are a major concern for worldwide health [1]. In tuberculosis and GRAM+ infection, Type I interferon (Type I IFN) has been shown to play a pathological role [2, 3]. Plasmacytoid dendritic cells (pDCs) are known to produce high amounts of Type I IFN in response to viral sensing [4, 5]. It is reported that pDCs are able to respond to GRAM+ bacteria. They can be recruited at the site of the infection and are enriched in patient TB lymph nodes but their role in the disease is still poorly investigated [6, 7, 8]. How pDCs sense GRAM+ bacteria is still debated. pDCs express few pathogen pattern recognition receptors, i.e., TLR7 and TLR9 localized in the endosomes and capable of sensing nucleic acid [9]. pDCs also express a range of cytosolic sensors, either at steady state, such as the helicases DHX9/DHX36 [10], or following innate activation, such as RIG I [11]

GRAM+ bacteria express lipoproteins on their surface membrane, which play an important role in their survival and pathogenicity [12]. Bacterial lipoproteins are recognized by TLR2/1 and induce activation and maturation in dendritic cells [13]. TLR2 knock-out mice are more susceptible to mycobacterial infection but *Mycobacterium tuberculosis* is able to hijack TLR2 signaling to enhance its survival in the host [14]. T-helper (Th) 1 is the protective response in TB and in many GRAM+ bacterial infections, while Th2/T regulatory (Treg) have been shown to

promote the disease [¹⁵]. Bacterial pathogens may alter this balance to allow their immune escape through a mechanism still not fully understood. [¹⁶]

Materials and Methods

Blood samples and cell isolation

Buffy coats from healthy human donors were obtained from Etablissement Français du Sang, Paris, Saint-Antoine Crozatier and Saint Louis blood bank. Tonsils were recovered from Hôpital Necker-Enfant malade. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density gradient centrifugation (Ficoll-Paque; GE Healthcare). pDCs and CD11c⁺ DCs were isolated by a first step of total DC enrichment (EasySep human Pan-DC Enrichment kit; Stem cell) followed by FACS sorting as Lineage⁻CD11c⁻CD4⁺ to a 99% purity [¹⁷]. Human naïve CD4 T cells were isolated from PBMC by negative selection (naïve CD4 T cell isolation kit; Miltenyi Biotec) to a >98% purity.

Cells were stained with FITC anti-CD3 (BD), FITC anti-CD14 (BD), FITC anti-CD16 (BD), FITC anti-CD19 (Miltenyi), PECy7 anti-CD11c (BD), VioGreen anti-CD4 (Miltenyi), PE anti-TLR1 (eBioscience), AlexaFluor700 anti-TLR2.

pDC culture

pDCs were cultured in RPMI 10% FBS at 10⁶ cells/ml in the presence of Influenza A/PR/8/34 (H1N1) 82 HA/ml (Charles River Laboratories), PAM3CSK4 1 µg/ml and 10 µg/ml (Invivogen), 10 ng/mL GM-CSF, 0,1 µg/mL LPS (Invivogen), 100 µg/mL heat-killed *Mycobacterium tuberculosis* (Invivogen), MOI 1 heat-killed *Staphylococcus aureus* (Invivogen), MOI 10 heat-killed *Listeria monocytogenes*. Blocking experiments were performed by pre-treating pDCs 1 hour before stimulation with: 1µM CU-CPT22 (Merck-Millipore), Human TLR1 Neutralizing antibody - Monoclonal Mouse IgG1 (Invivogen), Human TLR2 Detection and Neutralizing antibody - Monoclonal Human IgA2 (Invivogen), Mouse IgG1 isotype control antibody (Invivogen), Human IgA2 Isotype Control (Invivogen).

After culture, cells were stained with 4,6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich) that was added before acquisition to exclude dead cells. pDCs were stained with the following antibodies: AF700 anti-HLA-DR (biolegend), APC anti-ICOSL (R&D), PE anti-CD86 (BD), FITC anti-CD80 (PE), FITC anti-CD40 (B), Percp5.5 anti-CD83 (ebioscience), Percp5.5 anti PD-L1 (ebioscience). Isotype-matched antibodies were used as control. Cells were analyzed on a flow cytometer (BD Fortessa and BD LSRII) and data were processed using FlowJo software (Tree Star, Inc.).

Real Time quantitative RT-PCR

Total RNA was extracted from freshly isolated, 1-hour PAM3CSK4-activated pDCs, freshly isolated CD11c⁺ DCs, and HeLa cells, using RNeasy Micro kit (Qiagen) and processed as described. [18] The following probes (life technology) were used: TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR9, TLR10, B2M, GAPDH, RPL34

pDC-T cell cocultures

CD4 naïve T cells were stained with 5-(and 6)-Carboxyfluorescein diacetate succinimidyl ester (CFSE) (eBioscience). CD4 naïve T cells were cultured for 6 days with allogeneic activated pDCs stimulated (GRAM⁺ bacteria treated, PAM3CSK4 in combination with anti-TLRs antibody) at a 5:1 ratio as previously described [19]. After coculture, T-cell expansion was determined by cell counting, and the percentage of dividing cells was determined by flow-cytometer (BD LSR II). Supernatants were collected after 24 hours of polyclonal restimulation with anti-CD3/CD28 microbeads (Dyna). Cytokine measurement was performed by Cytometric Bead Array Flex Set (BD Biosciences). Acquisition was performed in a flow cytometer (BD LSR II) and data were analyzed using Fcap array (BD).

Intracellular Staining

CD4 naïve T-cells were cultured for 4 days with allogeneic activated pDCs (PAM3CSK4 in combination with anti-TLRs antibody). T-cells were stained with ZombieNir fixable kit (Biolegend) before surface staining, fixation and permeabilization (FOXP3 Fix/Perm buffers, eBioscience). Cells were then stained with, APC anti BCL-6 (BD), PercP55 anti Tbet (BD), Pcy7 anti GATA3, APC anti FoxP3 (eBioscience). Isotype-matched antibodies were used as control. Cells were analyzed on a flow cytometer (BD Fortessa and BD LSR II) and data were processed using FlowJo software (Tree Star, Inc.).

Phosphofacs

pDCs were treated during 4 hours with medium, PAM3CSK4 (in combination with neutralizing antibody as described before) and influenza virus. Cells were fixed with Fix Buffer I (BD) and permeabilized with Perm Buffer III (BD). Cells were stained with PE anti-p-AKT (BD), PECy7 anti-p-p65 (BD), PE anti-p-p38 (cell signal). Isotype-matched antibodies were used as control. Cells were analyzed on a flow cytometer (BD LSR II) and data were processed using FlowJo software (Tree Star, Inc.).

Statistical Analysis

Statistical analyses were performed to compare the different conditions by Wilcoxon paired test using Prism (GraphPad Software). Statistical significance was considered for p value <0.05

Results and discussion:

Human pDCs express functional TLR2 and TLR1 in order to respond to bacterial lipoproteins

In order to investigate how pDCs sense GRAM+ bacteria, we screened steady state pDCs TLRs mRNA expression. We noticed the expression of TLR1, TLR2 (basal level), TLR6, TLR10 (FIG S1A). We monitored pDCs TLR2 and TLR1 expression following lipoproteins stimulation in comparison with HeLa cells (negative control) and CD11c+ DCs (positive control). pDCs maintained a stable TLR1 expression following stimulation, and increased their TLR2 expression in comparable manner to CD11c+ DCs (FIG 1A and B). We confirmed pDCs expression of surface TLR1 and TLR2 at the protein level using flow cytometry (FIG 1C and FIG S1B).

To address the functionality of TLR1 and 2 on pDCs, we investigated pDC response to PAM3CSK4 (PAM3), a bacterial lipoprotein used as a prototypical TLR1/2 ligand. We observed an upregulation of co-stimulatory molecules and MHC-II expression on the surface (FIG 1D, S1C and D) to an extent comparable to known pDC stimuli, such as GM-CSF and influenza virus.^[20] PAM3 induced the secretion of high levels of type I IFN and pro-inflammatory cytokines, although to a slightly lesser extent than with the influenza virus (Flu) (FIG 1E). Although it is reported that TLR2 in inflammatory monocytes can be endocytosed and activate IRF7 in response to a viral ligand, these results are the first report of Type I Interferon induction by TLR2 in response to a bacterial ligand ^[21]. pDCs produce Granzyme B in the active form and are capable of inducing T-cell proliferation suppression in a perforin independent manner ^[22]. Lozza et al. have shown that pDCs in TB patient lymph node produce GrB; our data suggest that GrB can be induced by bacterial lipoproteins. We stimulated pDC purified from tonsils, a site of frequent encounter with GRAM+ bacteria, with bacterial lipoproteins to find out whether they respond in the same way as blood pDCs (FIG S1E). Tonsil pDCs up-regulate surface co-stimulatory molecules to the same extent as blood pDCs do. These data suggest that pDCs from blood and from physiological bacterial interfaces functionally respond to bacterial lipoproteins. TLR1, TLR6 and TLR10 are known to be able to heterodimerize with TLR2 following ligand stimulation. ^[23] TLR6 expression suggests that human pDCs can respond to diacyetyl-lipoproteins (TLR2/6 ligands).

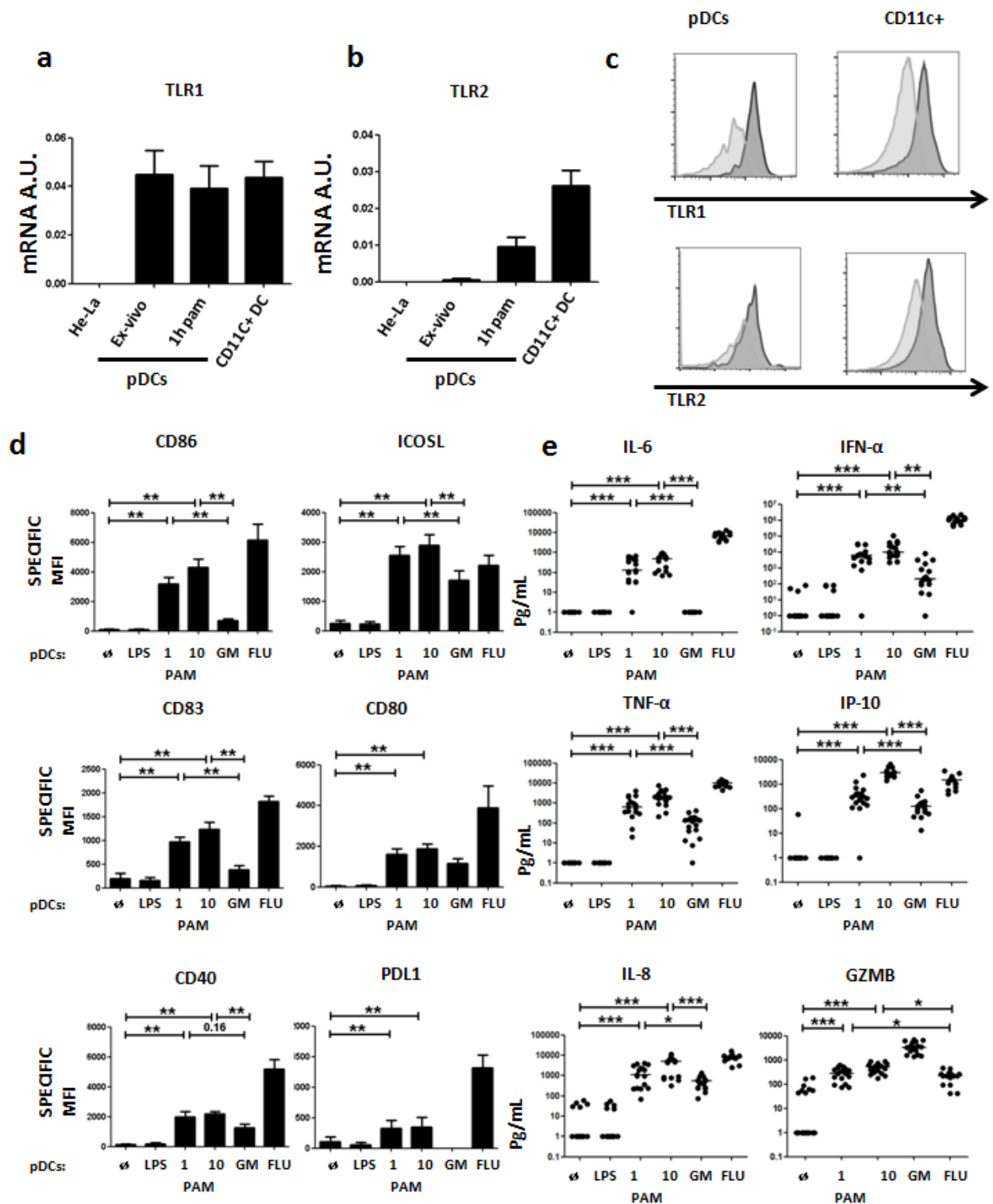


Figure 1. : Human pDCs express TLR2/1 and respond to PAM3

A-B. Real time RT-PCR from total mRNA from sorted human pDCs (and after 1 hour activation with PAM3CSK4), CD11c+ and HeLa cells. Results were normalized on three housekeeping genes. Results include 5 donors. **A.** TLR1 mRNA expression **B.** TLR1 mRNA expression. **C.** pDCs and CD11c+ were stained in freshly isolated PBMCs with anti-TLR1 and anti-TLR2 antibody, respective cognate isotype. **D-**

E. Sorted human pDCs were cultured with only medium (∅), LPS, 1 and 10 µg/mL PAM3CSK4 (PAM3), GM-CSF (GM), influenza virus (FLU), during 24 hours. **D.** Surface expression of co-stimulatory or co-inhibitory molecules from activated pDCs. Results include the mean of 9 donors. **E.** Cytokines secretion by pDCs. Results include the mean of 17 donors. $p<0.05$; $**p<0.01$; $***p<0.001$ (Wilcoxon test)

pDCs sense and respond to GRAM+ bacteria through TLR2/1 pathway

We next questioned whether, in addition to purified lipoprotein, pDCs could respond similarly to whole GRAM+ bacteria (FIG 2A, B). pDCs were stimulated with three different heat-killed GRAM+ bacteria: *Mycobacterium tuberculosis*, *Staphylococcus aureus* and *Listeria monocytogenes*. While pDCs activation by *S. aureus* is reported, whether pDCs can respond to *M. tuberculosis* is still debated. [24] We observed a strong bacteria-induced pDC activation, with both costimulatory molecule upregulation, and type I IFN production, to similar levels compared to PAM3 (FIG 2A). In addition, GRAM+ stimulated pDCs secrete high amount of type I IFN (FIG 2B). In parallel, we used a competitive antagonist of bacterial lipoprotein (CU-CPT22) to assess if GRAM+ bacteria pDCs sensing was TLR2/1-mediated (FIG 2A and B, S2A-C). [25] TLR2/1 blocking decreased bacteria-induced co-stimulatory molecule expression, and almost abrogated type I IFN production (FIG2B). Thus, pDCs responded to whole Gram+ bacteria in a TLR2/1-dependent manner.

T-cell priming is an important adaptive function of activated pDCs. [26] pDCs primed with GRAM+ bacteria were cultured with heterologous naïve CD4+ T-cells for 6 days, and the influenza virus was used as a positive control for pDC activation [27]. Bacteria-primed pDCs were capable of inducing T-cell expansion (FIG 2C) and proliferation (FIG 2D) comparable to viral stimulation. We further investigated whether T-cell activation was associated with Th-specific cytokine patterns. After 6 days of co-culture, T-cells were polyclonally re-stimulated. GRAM+ bacteria-activated pDCs induced secretion of IL-4, IFN-γ indicative, respectively, of a TH2 and a TH1 profile. We further noticed the presence of IL-10 produced by Tregs. We noticed a mixed Th profile (TH1, TH2 and Tregs).

In the last few years, different attempts to develop a vaccine against *S. aureus* toxins and immune evasion factors have failed. [28] Successively, lipoproteins have been considered promising candidates. Besides, vaccines in combination with TLR7 ligand show a boost in the protective immunity [29]. Our results suggest the possible role of pDCs in vaccine efficacy, considering their capacity to respond to lipoproteins, high TLR7 expression and capacity to prime T-cells in response to GRAM+ bacteria.

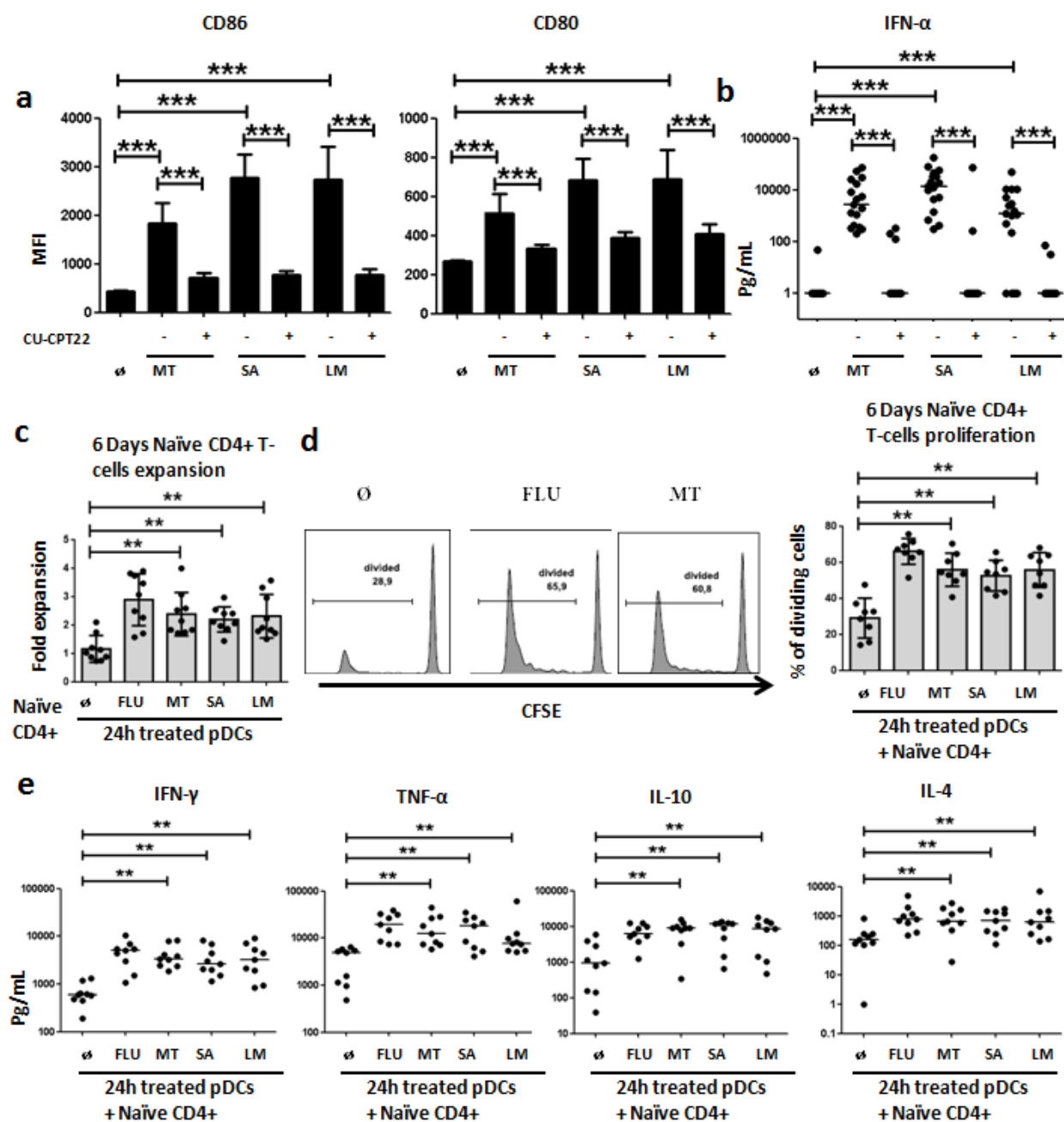


Figure 2. : pDCs sense different GRAM+ bacteria through TLR2/1 pathway

A-B. Sorted human pDCs were cultured during 24 hours with only medium (\emptyset), heat-killed *Mycobacterium tuberculosis* (MT), heat-killed *Staphylococcus aureus* (SA), heat-killed *Listeria monocytogenes* (LM) in the presence (+) or absence (-) of CU-CPT22. **A.** Surface expression of co-stimulatory molecules from activated pDCs. Results include the mean of 27 independent donors. **B.** Cytokines secretion by pDCs. Results include the mean of 17 independent donors. **C-D.** Heterologous CD4+ naïve expansion and percentage of dividing living cells after 6 days co-culture with 24 hours GRAM+ stimulated pDCs. Influenza virus (FLU) activated pDCs was used as a control. Results include the mean of 9 donors. **E.** T-helper cytokine pattern from GRAM+ pDCs activated T-cells co-culture. Cytokines were measured after 24h polyclonal re-stimulation of the T-cells. Results include the mean of

9 independent donors. $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Wilcoxon test).

TLR2 and TLR1 play a different role in the pDCs lipoprotein response

In order to investigate the differential contribution of TLR1 and TLR2 in mediating pDC response to bacterial lipoproteins, we alternately blocked the two receptors with specific antibodies. TLR1 blocking affected the co-stimulatory molecule expression (CD80, CD86 and ICOSL) (FIG 3A and S3A), while TLR2 did not have any impact at this level. TLR1 blocking almost abolished pro-inflammatory cytokine secretion (IL-6 and TNF- α) (FIG 3B). Conversely, TLR2 blocking inhibited type I IFN secretion, which was not affected by the following TLR1 blocking (FIG 3B). These results reveal a differential control of pDCs activation by each receptor. Considering that these molecules are also involved in the potential to induce T-cell polarization, we performed co-culture experiments with lipoprotein-treated pDCs and naïve T-cells, with and without blocking antibodies to TLR1 or TLR2. TLR1 blocking affected T cell expansion and proliferation (FIG 3C and S3B). We analyzed the TH cytokines pattern after polyclonal re-stimulation. We did not notice difference in the TH1-like profile (IFN- γ , IL-3, FIG 3D and S3D); TLR1 blocking in pDCs, however, impacted on the TH2-like profile (IL-13, IL-4, IL-5) (FIG 3D and S3D). TLR1 blocking also diminished IL-10 production by Th cells, suggesting a decrease in Treg generation. We further noticed that TLR1 blocking reduced IL-9 secretion by Th cells. IL-9 production can be associated to allergy, suggesting a previously undescribed link between TLR1 and a pro-allergic Th-cells phenotype. pDCs play a regulatory role in multiple sclerosis through the induction of IL-9 in Tcells to counterplay the pathogenic TH17 [³⁰]. These reports open questions about the role of bacteria, surface TLRs and pDCs in allergy and multiple sclerosis. TH1 cells play a protective role in GRAM+ bacterial infection, while TH2 cytokines and Tregs are linked to the bacterial immune evasion. After 4 days of pDC T-cells co-culture we performed intracellular staining for TH master regulator genes to better characterize the T-helper subset induced. We confirmed that TLR1/TLR2 blocking did not affect Tbet induction (hence TH1 polarization); conversely, TLR1 blocking impacted on GATA3, FOXP3 (confirming its impact on TH2 and GATA3 polarization). TLR1 blocking also affected BCL-6 expression, involved in Tfh generation. [³¹] (FIG 3E and S3C) Moreover, pDCs are linked to autoimmune diseases such as SLE, where auto-antibodies are present. [³², ³³]

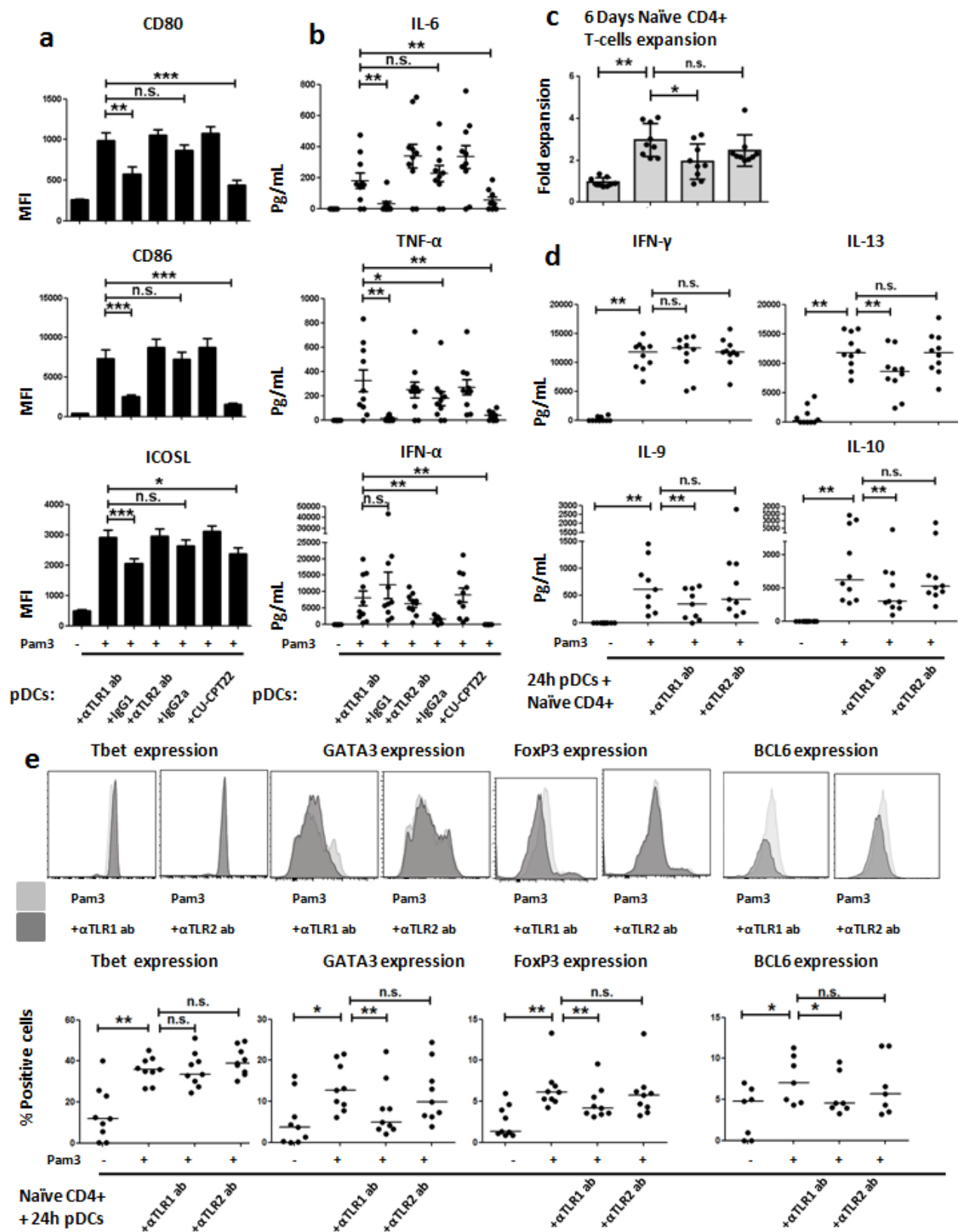


Figure 3. : pDCs differential TLR2 and TLR1 pDCs activation

A-B. Sorted human pDCs were cultured during 24 hours with only medium (∅) and PAM3CSK4 (PAM3)

in combination with TLR1 Neutralizing antibody (α TLR1 ab), TLR2 Neutralizing antibody (α TLR2 ab), IgG1 isotype control antibody (IgG1), IgA2 Isotype Control (IgG2a), CU-CPT22. **A.** Surface expression of co-stimulatory molecules from activated pDCs. Results include the mean of 17 independent donors. **B.** Cytokines secretion by pDCs. Results include the mean of 10 independent donors. **C-D.** Heterologous CD4⁺ naïve expansion after 6 days co-culture with 24 hours PAM3CSK4 pDCs (in the presence/absence of blocking antibodies). Results, which include the mean of 9 independent donors, were measured after 24h polyclonal re-stimulation of the T-cells. **D.** T-helper cytokine pattern from PAM3CSK4 (in combination with neutralizing antibody) activated T-cells co-culture. Cytokines were measured after 24h polyclonal re-stimulation of the T-cells. Results include the mean of 9 independent donors. **E.** Percentage of T-helper master regulator expression from PAM3CSK4 pDCs (in presence/absence of neutralizing antibodies) T-cells co-culture. Intracellular FACS was performed after 4 days of co-culture. Results include the mean of 9 independent donors for Tbet, GATA3, FoxP3. Results include the mean of 7 independent donors for BCL-6 $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Wilcoxon test).

TLR2 and TLR1 induce different pathway activation in response to bacterial lipoprotein

We further questioned the mechanism behind these differences in the pDCs activation. In pDCs it was shown that MAPK and NF- κ B pathway activation led to co-stimulatory expression and pro-inflammatory cytokine release, while PI3K signaling controlled Type I IFN induction [³⁴]. We performed phosphoFACS to investigate which pathways were activated by bacterial lipoproteins in pDCs. Stimulation with PAM3 led to p38, p65, and AKT phosphorylation (FIG 4A and B). These results suggested that MAPK, NF- κ B and PI3K were being activated. We wondered about whether the differences observed in the TLR blocking experiment were due to a diverse pathway engagement. The TLRs blocking showed that MAPK and NF- κ B pathways were TLR1-dependent, while the PI3K pathway was TLR2-dependent (FIG4C and S4A). These results explained the behavior observed. Co-stimulatory molecules and pro-inflammatory cytokines secretion could be linked to MAPK and NF- κ B pathway in pDCs, while type I IFN production required PI3K pathway activation. In pDCs, the endosomal TLRs signaling has been extensively studied: TLR7 and TLR9 in the early endosomes lead to IRF7 recruitment and Type I IFN induction, while after maturation to the late endosomes they activate MAPK and NF- κ B pathway [³⁵]. Since TLR2 activates the PI3K pathway it remains to evaluate whether the receptor has to be internalized to induce Type I interferon.

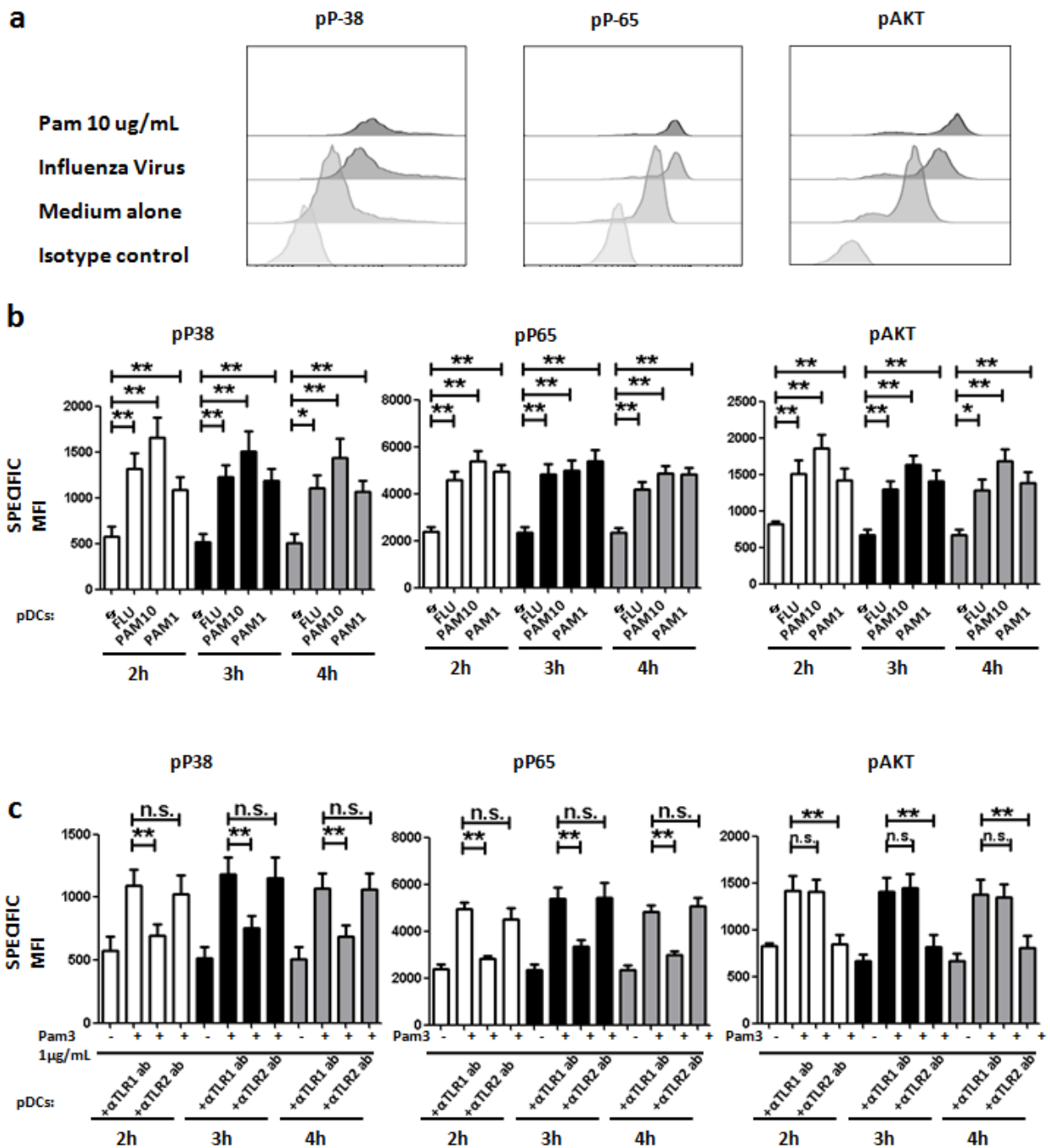


Figure 4. : pDCs differential TLR2 and TLR1 pDCs signaling

A-C. Sorted human pDCs were cultured during 4 hours with only medium (\varnothing) and PAM3CSK4 (PAM3) in combination with TLR1 Neutralizing antibody (α TLR1 ab), TLR2 Neutralizing antibody (α TLR2 ab), IgG1. Influenza virus (FLU) was used as control. **A** p38 MAPK (first panel), p65 NF- κ B (second panel), AKT PI3K (third panel) at 3 hours. **B-C.** p38 MAPK (first panel), p65 NF- κ B (second panel), AKT PI3K (third panel) at three different time points (2, 3, 4 hours). Results include the mean of 8 independent donors. $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Wilcoxon test)

Our results demonstrate that pDCs express functional TLR2 and TLR1 that mediate response to

bacterial lipoproteins and whole GRAM+ bacteria. We have provided the first demonstration of surface TLRs inducing Type I Interferon. This opens broad perspectives on the possible role of pDCs in bacterial diseases. Commensal microbe pDCs interactions seem to be involved in promoting the proliferation of ICOS+ Tregs to avoid colitis in the neo-adjuvants cancer treatment. [³⁶, ³⁷] The differences encountered in the activation mediated by TLR1 and TLR2 can help explain why these receptors can be implied in tolerance and autoimmunity or hijacked by bacteria. These data suggest that TLR1 is involved more in T-cell priming while TLR2 is more focused on alerting the innate immunity through Type I IFN control. In perspective, this also creates a manageable system that can be exploited in vaccine development, in the study of the role of type I IFN in bacterial infection, or as a tool for studying TH polarization.

References and notes

1. Mario Raviglione and Giorgia Sulis, "Tuberculosis 2015: Burden, Challenges and Strategy for Control and Elimination," *Infectious Disease Reports* 8, no. 2 (June 24, 2016), <http://www.pagepress.org/journals/index.php/idr/article/view/6570>.
2. Matthew P. R. Berry et al., "An Interferon-Inducible Neutrophil-Driven Blood Transcriptional Signature in Human Tuberculosis," *Nature* 466, no. 7309 (Agosto 2010): 973–77, doi:10.1038/nature09247.
3. Rosane M. B. Teles et al., "Type I Interferon Suppresses Type II Interferon–Triggered Human Anti-Mycobacterial Responses," *Science* 339, no. 6126 (March 22, 2013): 1448–53, doi:10.1126/science.1233665.
4. Melissa Swiecki and Marco Colonna, "The Multifaceted Biology of Plasmacytoid Dendritic Cells," *Nature Reviews Immunology* 15, no. 8 (Agosto 2015): 471–85, doi:10.1038/nri3865.
5. Marina Cella et al., "Plasmacytoid Monocytes Migrate to Inflamed Lymph Nodes and Produce Large Amounts of Type I Interferon," *Nature Medicine* 5, no. 8 (Agosto 1999): 919–23, doi:10.1038/11360.
6. Paula Michea et al., "Epithelial Control of the Human pDC Response to Extracellular Bacteria," *European Journal of Immunology* 43, no. 5 (Maggio 2013): 1264–73, doi:10.1002/eji.201242990.
7. Laura Lozza et al., "Communication between Human Dendritic Cell Subsets in Tuberculosis: Requirements for Naive CD4+ T Cell Stimulation," *Microbial Immunology* 5 (2014): 324, doi:10.3389/fimmu.2014.00324.

8. Isabelle Bekeredjian-Ding et al., "Plasmacytoid Dendritic Cells: Neglected Regulators of the Immune Response to Staphylococcus Aureus," *Microbial Immunology* 5 (2014): 238, doi:10.3389/fimmu.2014.00238.
9. Norimitsu Kadowaki et al., "Subsets of Human Dendritic Cell Precursors Express Different Toll-like Receptors and Respond to Different Microbial Antigens," *The Journal of Experimental Medicine* 194, no. 6 (September 17, 2001): 863–70, doi:10.1084/jem.194.6.863.
10. Taeil Kim et al., "Aspartate-Glutamate-Alanine-Histidine Box Motif (DEAH)/RNA Helicase A Helicases Sense Microbial DNA in Human Plasmacytoid Dendritic Cells," *Proceedings of the National Academy of Sciences* 107, no. 34 (August 24, 2010): 15181–86, doi:10.1073/pnas.1006539107.
11. Attila Szabo et al., "TLR Ligands Upregulate RIG-I Expression in Human Plasmacytoid Dendritic Cells in a Type I IFN-Independent Manner," *Immunology and Cell Biology* 92, no. 8 (Settembre 2014): 671–78, doi:10.1038/icb.2014.38.
12. Minh Thu Nguyen and Friedrich Götz, "Lipoproteins of Gram-Positive Bacteria: Key Players in the Immune Response and Virulence," *Microbiology and Molecular Biology Reviews* 80, no. 3 (September 1, 2016): 891–903, doi:10.1128/MMBR.00028-16.
13. Mi Sun Jin et al., "Crystal Structure of the TLR1-TLR2 Heterodimer Induced by Binding of a Tri-Acylated Lipopeptide," *Cell* 130, no. 6 (Settembre 2007): 1071–82, doi:10.1016/j.cell.2007.09.008.
14. Xiaowen Yu, Jie Zeng, and Jianping Xie, "Navigating through the Maze of TLR2 Mediated Signaling Network for Better Mycobacterium Infection Control," *Biochimie* 102 (Luglio 2014): 1–8, doi:10.1016/j.biochi.2014.02.012.
15. Luke D. Jasenosky et al., "T Cells and Adaptive Immunity to Mycobacterium Tuberculosis in Humans," *Immunological Reviews* 264, no. 1 (March 1, 2015): 74–87, doi:10.1111/imr.12274.
16. Jonathan Kevin Sia, Maria Georgieva, and Jyothi Rengarajan, "Innate Immune Defenses in Human Tuberculosis: An Overview of the Interactions between Mycobacterium Tuberculosis and Innate Immune Cells," *Journal of Immunology Research* 2015 (2015): 1–12, doi:10.1155/2015/747543.

17. Norimitsu Kadowaki et al., "Natural Interferon α/β -Producing Cells Link Innate and Adaptive Immunity," *The Journal of Experimental Medicine* 192, no. 2 (July 17, 2000): 219–26, doi:10.1084/jem.192.2.219.
18. Elisabetta Volpe et al., "A Critical Function for Transforming Growth Factor-B, Interleukin 23 and Proinflammatory Cytokines in Driving and Modulating Human TH-17 Responses," *Nature Immunology* 9, no. 6 (Giugno 2008): 650–57, doi:10.1038/ni.1613.
19. M C Rissoan et al., "Reciprocal Control of T Helper Cell and Dendritic Cell Differentiation.," *Science (New York, N.Y.)* 283, no. 5405 (February 19, 1999): 1183–86, doi:10.1126/science.283.5405.1183.
20. Cristina Ghirelli, Raphaël Zollinger, and Vassili Soumelis, "Systematic Cytokine Receptor Profiling Reveals GM-CSF as a Novel TLR-Independent Activator of Human Plasmacytoid Predendritic Cells," *Blood* 115, no. 24 (June 17, 2010): 5037–40, doi:10.1182/blood-2010-01-266932.
21. Roman Barbalat et al., "Toll-like Receptor 2 on Inflammatory Monocytes Induces Type I Interferon in Response to Viral but Not Bacterial Ligands," *Nature Immunology* 10, no. 11 (November 2009): 1200–1207, doi:10.1038/ni.1792.
22. Bernd Jahrsdörfer et al., "Granzyme B Produced by Human Plasmacytoid Dendritic Cells Suppresses T-Cell Expansion," *Blood* 115, no. 6 (February 11, 2010): 1156–65, doi:10.1182/blood-2009-07-235382.
23. Jeroen van Bergenhenegouwen et al., "TLR2 & Co: A Critical Analysis of the Complex Interactions between TLR2 and Coreceptors," *Journal of Leukocyte Biology* 94, no. 5 (November 1, 2013): 885–902, doi:10.1189/jlb.0113003.
24. Laura Lozza et al., "Crosstalk between Human DC Subsets Promotes Antibacterial Activity and CD8+T-Cell Stimulation in Response to Bacille Calmette-Guérin," *European Journal of Immunology* 44, no. 1 (January 2014): 80–92, doi:10.1002/eji.201343797.
25. Kui Cheng et al., "Discovery of Small Molecule Inhibitors of the TLR1-TLR2 Complex," *Angewandte Chemie (International Ed. in English)* 51, no. 49 (December 3, 2012): 12246–49, doi:10.1002/anie.201204910.
26. Kadowaki et al., "Natural Interferon α/β -Producing Cells Link Innate and Adaptive Immunity." *The Journal of Experimental Medicine* 192, no. 2 (July 17, 2000): 219–26. doi:10.1084/jem.192.2.219.

27. Marina Cella et al., "Plasmacytoid Dendritic Cells Activated by Influenza Virus and CD40L Drive a Potent TH1 Polarization," *Nature Immunology* 1, no. 4 (Ottobre 2000): 305–10, doi:10.1038/79747.
28. Fabio Bagnoli, Sylvie Bertholet, and Guido Grandi, "Inferring Reasons for the Failure of Staphylococcus Aureus Vaccines in Clinical Trials," *Frontiers in Cellular and Infection Microbiology* 2 (2012), doi:10.3389/fcimb.2012.00016.
29. Fabio Bagnoli et al., "Vaccine Composition Formulated with a Novel TLR7-Dependent Adjuvant Induces High and Broad Protection against Staphylococcus Aureus," *Proceedings of the National Academy of Sciences* 112, no. 12 (March 24, 2015): 3680–85, doi:10.1073/pnas.1424924112.
30. Gabriella Ruocco et al., "T Helper 9 Cells Induced by Plasmacytoid Dendritic Cells Regulate Interleukin-17 in Multiple Sclerosis," *Clinical Science* 129, no. 4 (August 1, 2015): 291–303, doi:10.1042/CS20140608.
31. Di Yu et al., "The Transcriptional Repressor Bcl-6 Directs T Follicular Helper Cell Lineage Commitment," *Immunity* 31, no. 3 (Settembre 2009): 457–68, doi:10.1016/j.immuni.2009.07.002.
32. Sarah L. Rowland et al., "Early, Transient Depletion of Plasmacytoid Dendritic Cells Ameliorates Autoimmunity in a Lupus Model," *The Journal of Experimental Medicine* 211, no. 10 (September 22, 2014): 1977–91, doi:10.1084/jem.20132620.
33. Lars Rönnblom and Gunnar V. Alm, "A Pivotal Role for the Natural Interferon A–producing Cells (Plasmacytoid Dendritic Cells) in the Pathogenesis of Lupus," *Journal of Experimental Medicine* 194, no. 12 (December 17, 2001): F59–64, doi:10.1084/jem.194.12.f59.
34. Cristiana Guiducci et al., "PI3K Is Critical for the Nuclear Translocation of IRF-7 and Type I IFN Production by Human Plasmacytoid Predendritic Cells in Response to TLR Activation," *The Journal of Experimental Medicine* 205, no. 2 (February 18, 2008): 315–22, doi:10.1084/jem.20070763.
35. Roberto Lande and Michel Gilliet, "Plasmacytoid Dendritic Cells: Key Players in the Initiation and Regulation of Immune Responses," *Annals of the New York Academy of Sciences* 1183, no. 1 (January 2010): 89–103, doi:10.1111/j.1749-6632.2009.05152.x.

36. Jonathan M. Pitt et al., "Resistance Mechanisms to Immune-Checkpoint Blockade in Cancer: Tumor-Intrinsic and -Extrinsic Factors," *Immunity* 44, no. 6 (June 2016): 1255–69, doi:10.1016/j.immuni.2016.06.001.
37. Jonathan M. Pitt et al., "Fine-Tuning Cancer Immunotherapy: Optimizing the Gut Microbiome," *Cancer Research* 76, no. 16 (August 15, 2016): 4602–7, doi:10.1158/0008-5472.CAN-16-0448.

Acknowledgements

This work was supported by funding from INSERM (BIO2014-08), FRM, ANR-13-BSV1-0024-02, ANR-10-IDEX-0001-02 PSL* and ANR-11-LABX-0043, ERC (IT-DC 281987 and HEALTH 2011-261366) and CIC IGR-Curie 1428. S.R. was supported by MESR fellowship.

Supplementary Materials

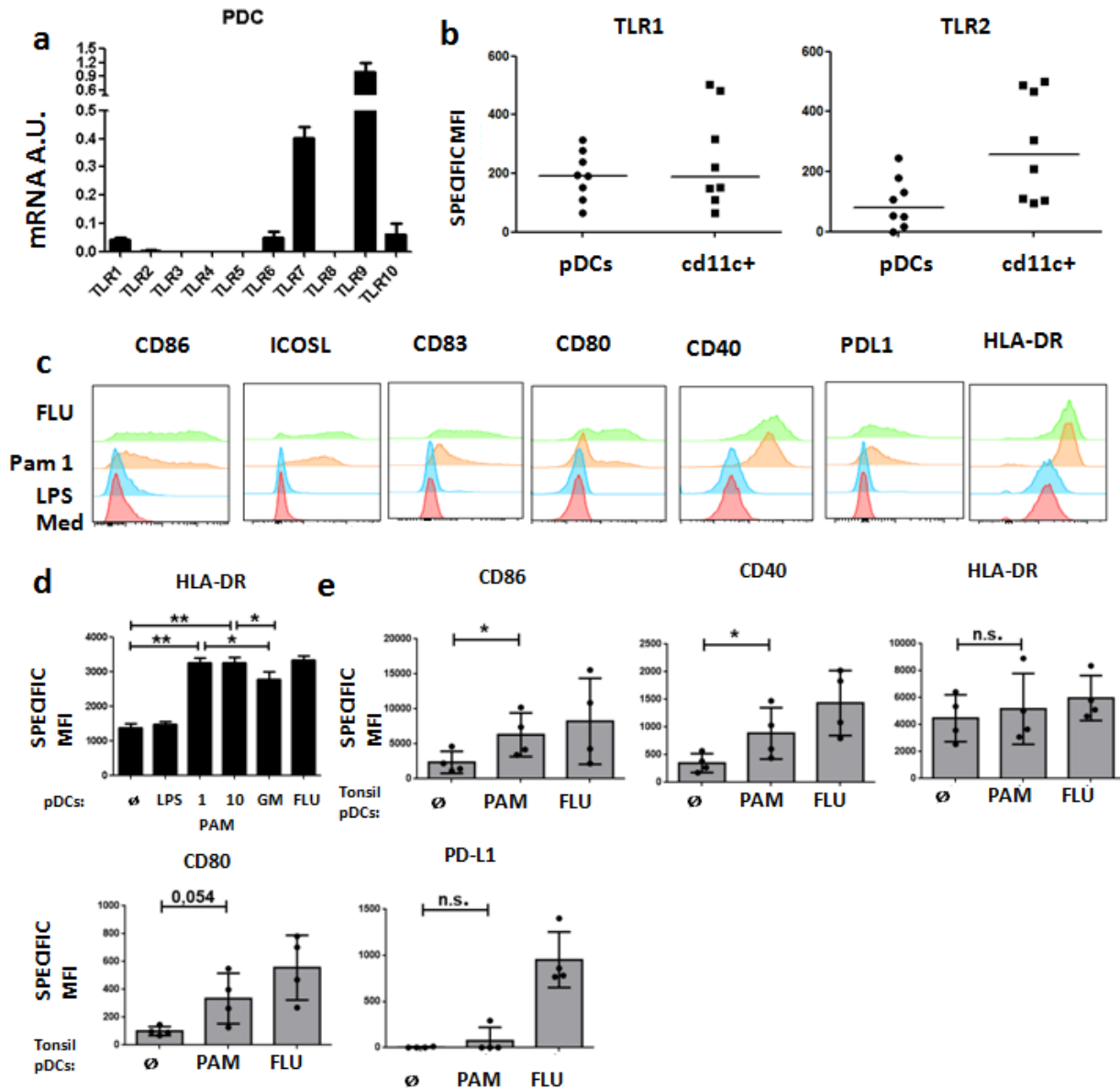


Figure S1. : pDCs from Human tonsil respond to Pam3

A. Real-time RT-PCR from total mRNA from sorted human pDCs. Results were normalized on three housekeeping genes. Results include 5 donors. **B.** pDCs and CD11c+ were stained in freshly isolated PBMCs with anti-TLR1 (left panel) and anti-TLR2 antibodies(right panel), respective cognate isotype. **C-D.** Sorted human pDCs were cultured with only medium (\emptyset), LPS, 1 and 10 $\mu\text{g}/\text{mL}$ PAM3CSK4 (PAM3), GM-CSF (GM), influenza virus (FLU), during 24 hours. **D** Surface expression of MHC-II complex from activated pDCs. Results include the mean of 9 donors. **E.** Sorted tonsil pDCs were stimulated during 24 hours with only medium (\emptyset), PAM3CSK4 (PAM3), influenza virus (FLU). Results include the mean of 4 donors. $p < 0.05$; $**p < 0.01$;

*** $p < 0.001$ (Wilcoxon test)

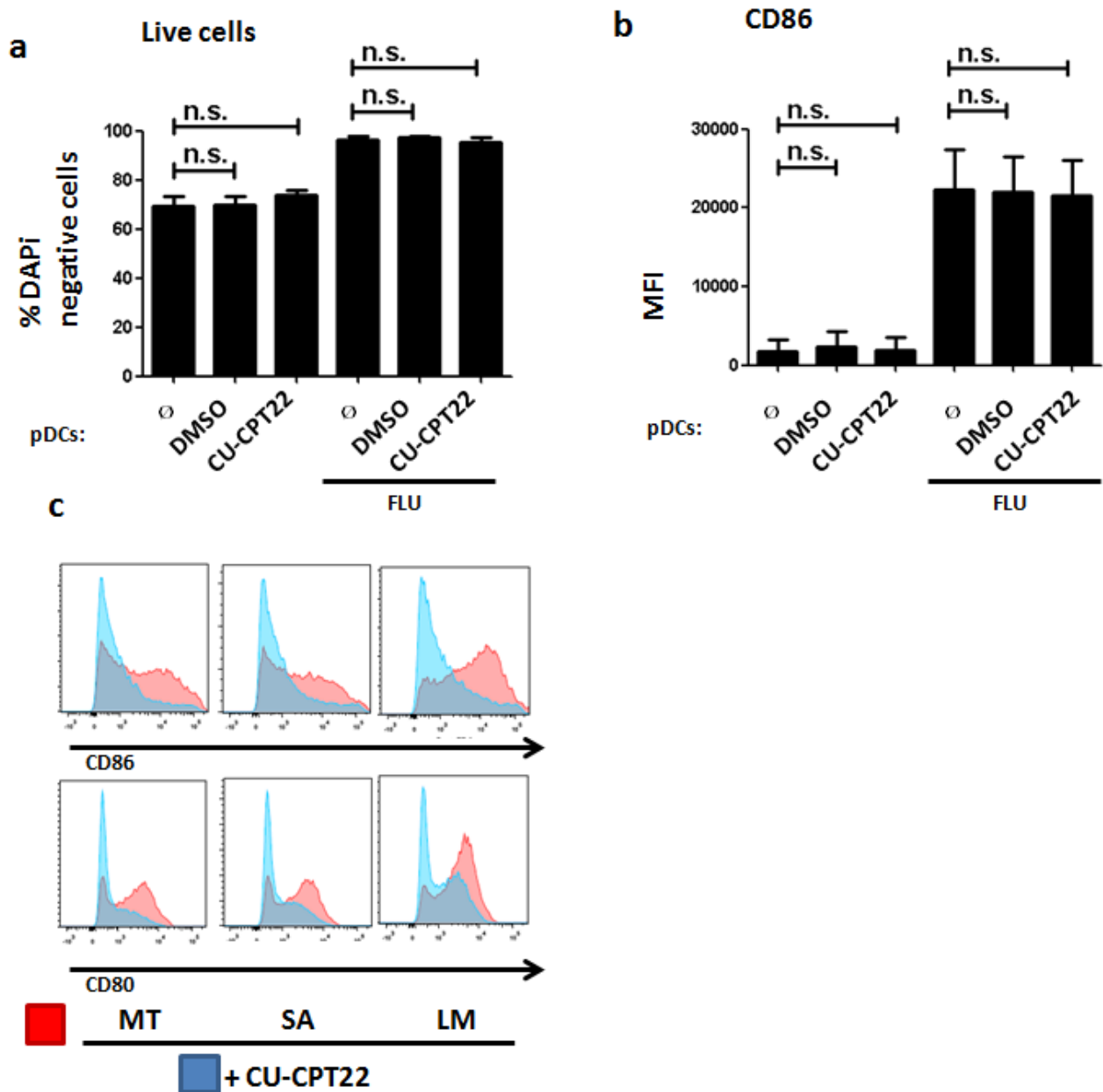


Figure S2. : pDCs sense different GRAM+ bacteria through TLR2/1 pathway

A-B. Sorted human pDCs were cultured during 24 hours with only medium (\emptyset), DMSO and CU-CPT22, influenza virus (in combination with DMSO and CU-CPT22) **A.** Cell viability as percentage of cells DAPI negative. Results include the mean of 4 independent donors. **B.** Surface expression of CD86 from treated pDCs. Results include the mean of 4 independent donors. **C.** Sorted human pDCs were cultured during 24 hours with only medium (\emptyset), heat-killed *Mycobacterium tuberculosis* (MT), heat-killed *Staphylococcus aureus* (SA), heat-killed *Listeria monocytogenes* (LM) in the presence (+) or absence (-) of CU-CPT22. Surface expression of co-stimulatory molecules from activated pDCs. $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Wilcoxon test)

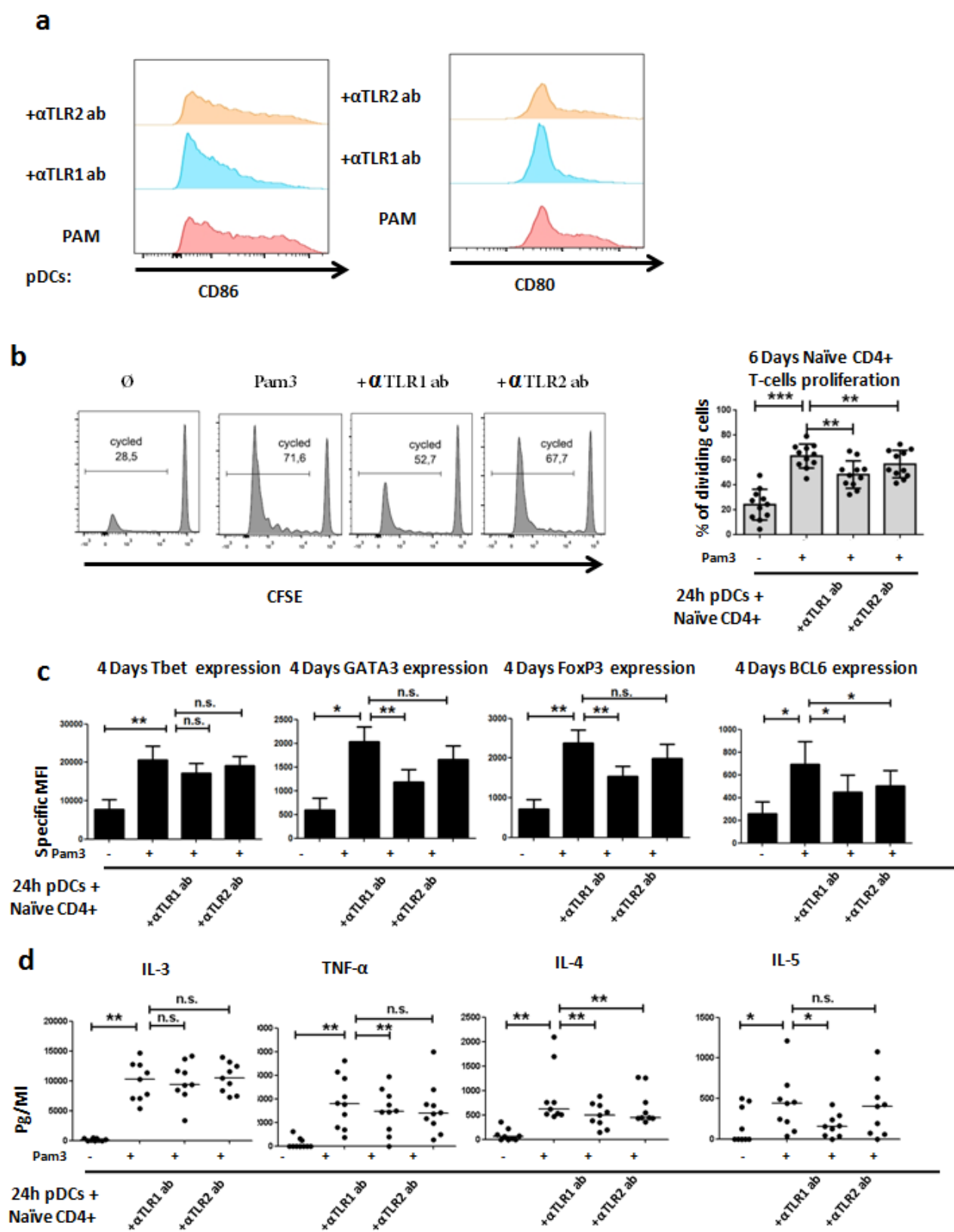


Figure S3. : pDCs differential TLR2 and TLR1 pDCs activation

A-B. Sorted human pDCs were cultured during 24 hours with only medium (\emptyset) and PAM3CSK4 (PAM3) in combination with TLR1 Neutralizing antibody (α TLR1 ab), TLR2 Neutralizing antibody (α TLR2 ab), IgG1 isotype control antibody (IgG1), IgA2 Isotype Control (IgG2a), CU-CPT22. **A.** Surface expression of co-stimulatory molecules from activated pDCs. **B.** Heterologous CD4⁺ naïve percentage of dividing cells after 6 days co-culture with 24 hours PAM3CSK4 pDCs (in the presence/absence of blocking antibodies). Results include the mean of 9 independent donors. **C.** Specific MFI of T-helper master regulator expression from PAM3CSK4 pDCs (in presence/absence of neutralizing antibodies) T-cells co-culture. Intracellular FACS was performed after 4 days of co-culture. Results include the mean of 9 independent donors for Tbet, GATA3 and FoxP3. Results include the mean of 7 independent donors for BCL-6 **D.** T helper cytokine pattern from PAM3CSK4 (in combination with neutralizing antibody) activated T-cells co-culture. Cytokines were measured after 24h polyclonal re-stimulation of the T-cells. Results include the mean of 9 independent donors. $p < 0.05$; $**p < 0.01$; $***p < 0.001$ (Wilcoxon test).

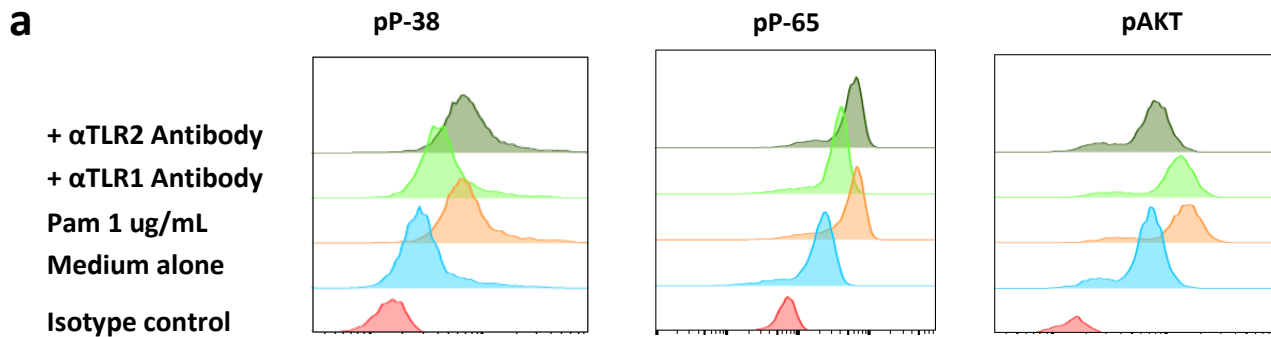


Figure S4. : pDCs differential TLR2 and TLR1 pDCs activation

A. Sorted human pDCs were cultured during 4 hours with only medium (\emptyset) and PAM3CSK4 (PAM3) in combination with TLR1 Neutralizing antibody (α TLR1 ab), TLR2 Neutralizing antibody (α TLR2 ab), IgG1. p38 MAPK (first panel), p65 NF- κ B (second panel), AKT PI3K (third panel) at 3 hours.

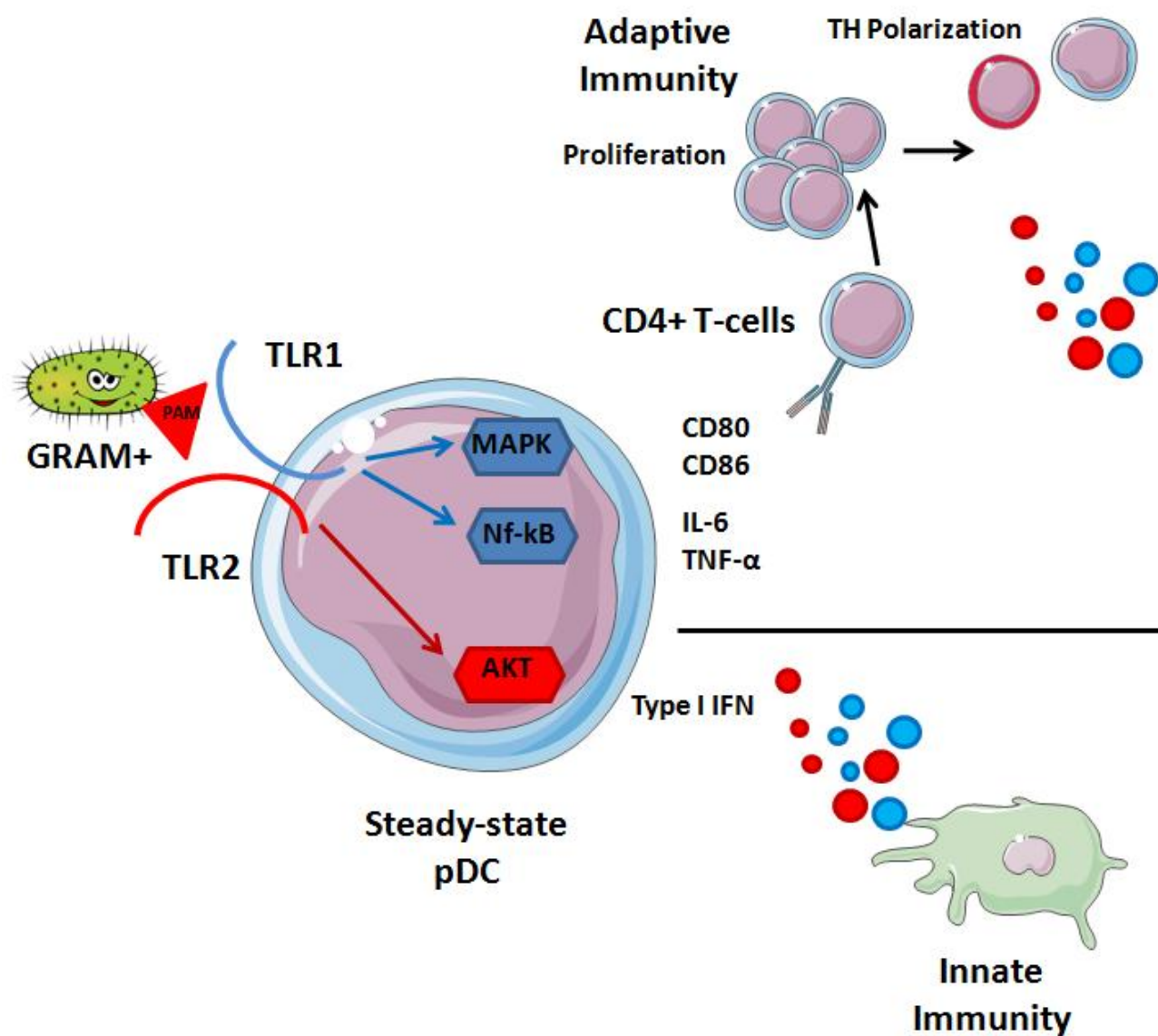


Figure S5. Graphical abstract

Our model suggests that human pDCs sense GRAM+ bacteria through the recognition of their bacterial lipoproteins. The LPs are recognized by TLR2/1 receptors, TLR2 is expressed in the presence of the ligand through a positive feedback. TLR1 engagement signals through MAPK and NF- κ B, regulating co-stimulatory expression (CD80, CD86, ICOSL) and pro-inflammatory cytokines secretion (IL-6, TNF- α). CD4+ expansion and proliferation are TLR1-dependent. TLR1 activation plays a role in the pDCs TH2 and Tregs induction. TLR2 specific controls Type I IFN secretion in pDCs response to bacterial lipoprotein through PI3K activation. TLR1 engagement leads to an adaptive function, while TLR2 engagement controls innate pDCs function.

ANNEXE DATA

Material and methods

Blood samples and cell isolation

Buffy coats from healthy human donors were obtained from *Etablissement Français du Sang, Paris, Saint-Antoine Crozatier* and *Saint Louis* blood bank. Tonsils were recovered from *Hôpital Necker-Enfant Malade*. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density gradient centrifugation (Ficoll-Paque; GE Healthcare). pDCs and CD11c⁺ DCs were isolated by a total DC enrichment (EasySep human Pan-DC Enrichment kit; Stem cell) followed by FACS sorting as Lineage⁻CD11c⁻CD4⁺ to a 99% purity. Human naïve CD4 T cells were isolated from PBMC by negative selection (naïve CD4 T cell isolation kit; Miltenyi Biotec) to a >98% purity. CD14⁺ monocytes were isolated with monocytes enrichment (CD14⁺ monocytes isolation kit; Miltenyi Biotec) to a >98% purity.

Cells were stained with FITC anti-CD3 (BD), FITC anti-CD14 (BD), FITC anti-CD16 (BD), FITC anti-CD19 (Miltenyi), PECy7 anti-CD11c (BD), VioGreen anti-CD4 (Miltenyi).

pDCs culture

pDCs were cultured in RPMI 10% FBS at 10⁶ cells/ml in the presence of Influenza A/PR/8/34 (H1N1) 82 HA/ml (Charles River Laboratories), PAM3CSK4 1 µg/ml and 10 µg/ml (Invivogen), 10 ng/mL GM-CSF, 0,1 µg/mL LPS (Invivogen), MOI 1 heat-killed *Staphylococcus aureus* (Invivogen), MOI 10 heat-killed *Listeria monocytogenes*. After culture, cells were stained with 4,6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich). DAPI was added before acquisition to exclude dead cells. Isotype-matched antibodies were used as control. Cells were analyzed on a flow cytometer (BD Fortessa and BD LSRII) and data was processed using FlowJo software (Tree Star, Inc.).

24 hour-pDCs subsets were cytopspined in parallel and dyed with Giemsa staining. Pictures were

taken with a CFW-1308C color digital camera (Scion Corporation) on a Leica DM 4000 B microscope, with a 100x /1.30 oil objective.

Real Time quantitative RT-PCR

Total RNA was extracted from freshly isolated pDCs (or 1 hour PAM3CSK4 activated), freshly isolated CD11c+ DCs and HeLa cells by using RNeasy Micro kit (Qiagen) and processed as described. The following probes (Life Technology) were used: GAPDH (Hs99999905_m1), B2M ,RPL34, TLR1 , TLR2, TLR3 , TLR4 , TLR5, TLR6, TLR7, TLR8, TLR9, TLR10

Intracellular Staining

pDCs were cultured for 24 hours with different stimuli. T cells were stained with ZombieNir fixable kit (Biolegend) before surface staining, fixation and permeabilization (FOXP3 Fix/Perm buffers, eBioscience). Cells were then stained with, APC antiGrB. Isotype-matched antibodies were used as control. Cells were analyzed on a flow cytometer (BD Fortessa and BD LSR II) and data were processed using FlowJo software (Tree Star, Inc.).

Statistical Analysis

Statistical analysis was performed among the different conditions by Wilcoxon paired test using Prism (GraphPad Software). Statistical significance was considered for p value <0.05.

Results

Quantitative RT-PCR was performed to monitor TLRs mRNA expression of different blood populations: plasmacytoid dendritic cells (pDCs, showed before), CD11c+ DCs and monocytes.

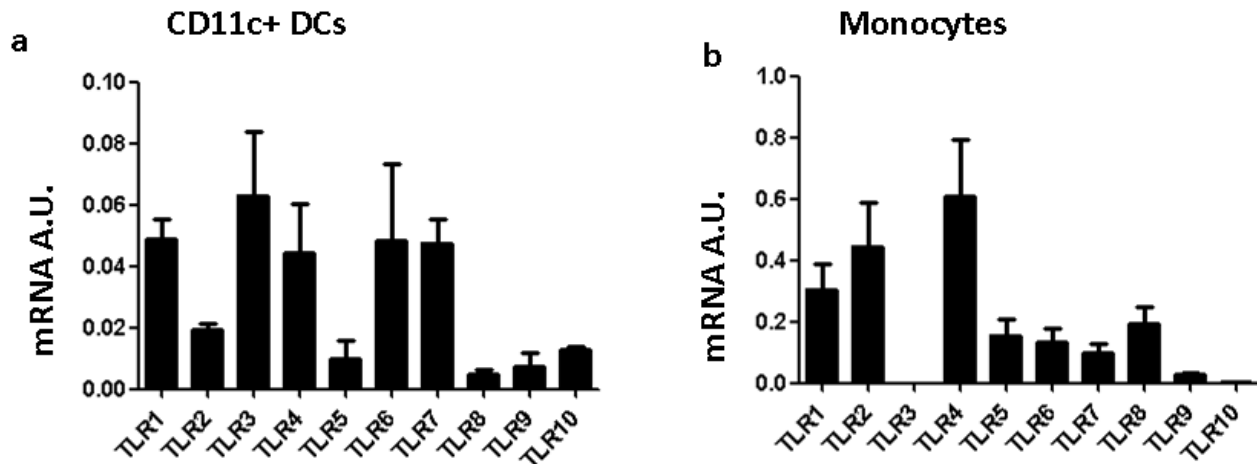


Figure 21: TLRs expression of different blood populations.

A-B. Real-time RT-PCR from sorted human CD11c+ DCs and isolated monocytes total mRNA. Results were normalized to three housekeeping genes. Results include 5 donors. **A.** CD11c+ DCs TLRs mRNA expression **B.** Monocytes TLRs mRNA expression.

pDCs, CD11c+ DCs and monocytes from the same human healthy donor (in order to reduce biological variability) were treated with different stimuli. pDCs lack TLR3, TLR4, TLR5 and TLR8 expression and do not respond to LPS (TLR4 ligand) or to TLR5 (ligand). pDCs do not respond to Type I IFN stimulation, even when expressing the IFNA receptor. CD11c+ DCs are responsive to almost all stimuli. Nevertheless, we noticed TLR7 mRNA expression in monocytes, which respond to loxoribine (syntethic TLR7 agonist).

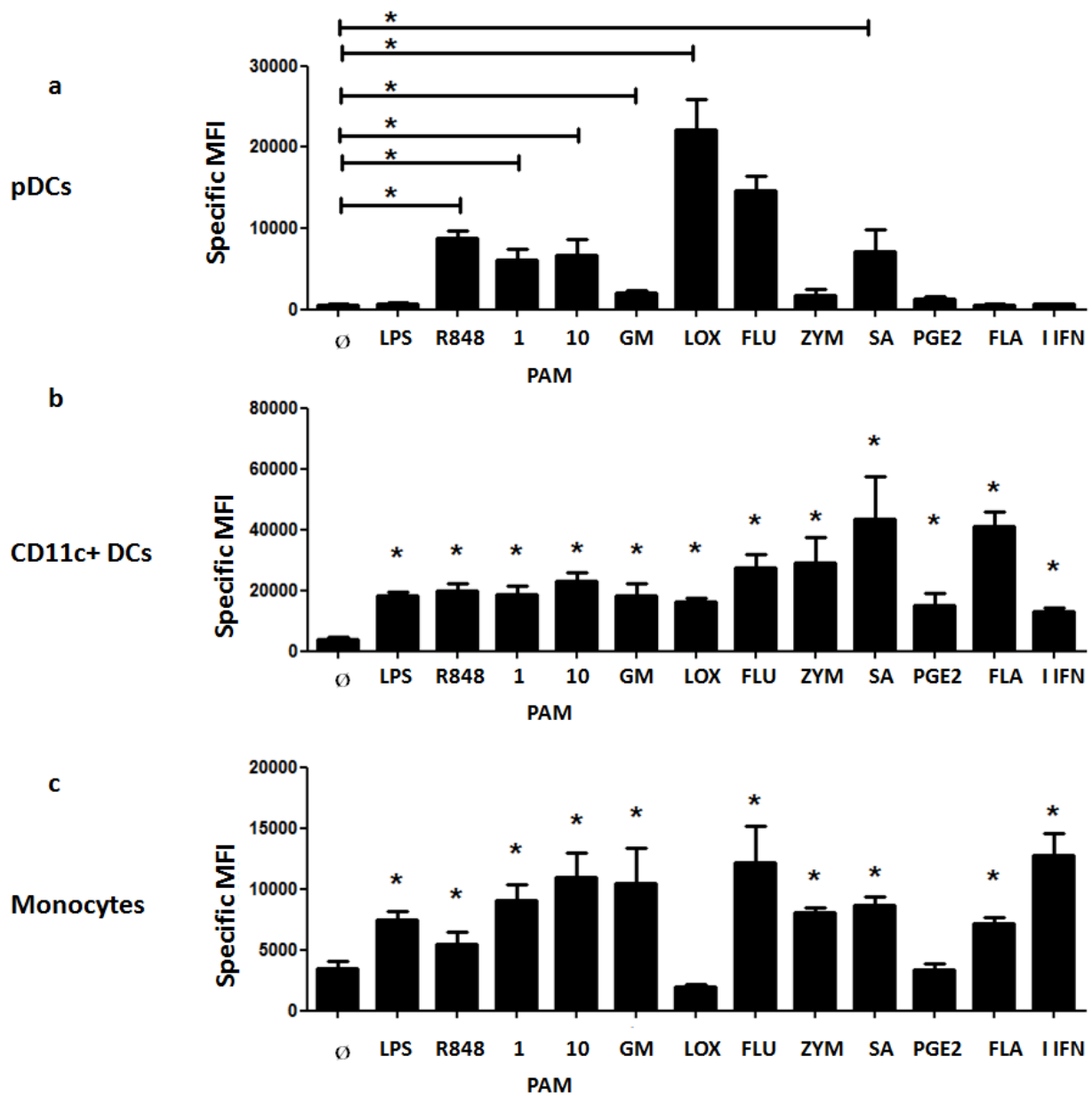


Figure 22: Comparison of stimuli response pattern among different human blood subpopulations

A-C. CD86 surface expression of different human blood subpopulations. Sorted human pDCs, sorted human CD11c+ DCs and enriched monocytes were treated with medium (Ø), LPS, R848, PAM3CKS4 (Pam3), GM-

CSF (GM), Loxoribine (LOX), influenza virus (FLU), Zymosan (ZYM), *S. aureus* (SA), Prostaglandin 2 (PGE2), Flagellin (FLA), Type I interferon (I IFN). **a.** Plasmacytoid DCs **b.** CD11c+ DCs **c.** Monocytes. Results include 5 independent donors. $p < 0.05$; $**p < 0.01$; $***p < 0.001$ (Wilcoxon test)

Having observed that pDCs respond to TLR2/1 ligand while expressing a basal level of TLR2, we investigated whether stimulation could alter the TLRs expression. We noticed that the same stimulation alters the various TLRs expression differently. Pam3, as shown in the previous chapter, induces up-regulation of TLR2 and a slight increase of TLR7 expression. Conversely, it reduces the expression of TLR9. Influenza virus increases TLR7's and abrogates TLR9's expression. GM-CSF reduces the expression of TLR9, while R848 (a synthetic agonist of TLR7 and TLR8) almost abolishes its expression. Interestingly, the stimulation of pDCs does not affect TLR4 (in the figure below) and TLR3, TLR5, TLR8 expression (data not shown), as none of the stimuli tested induced their expression.

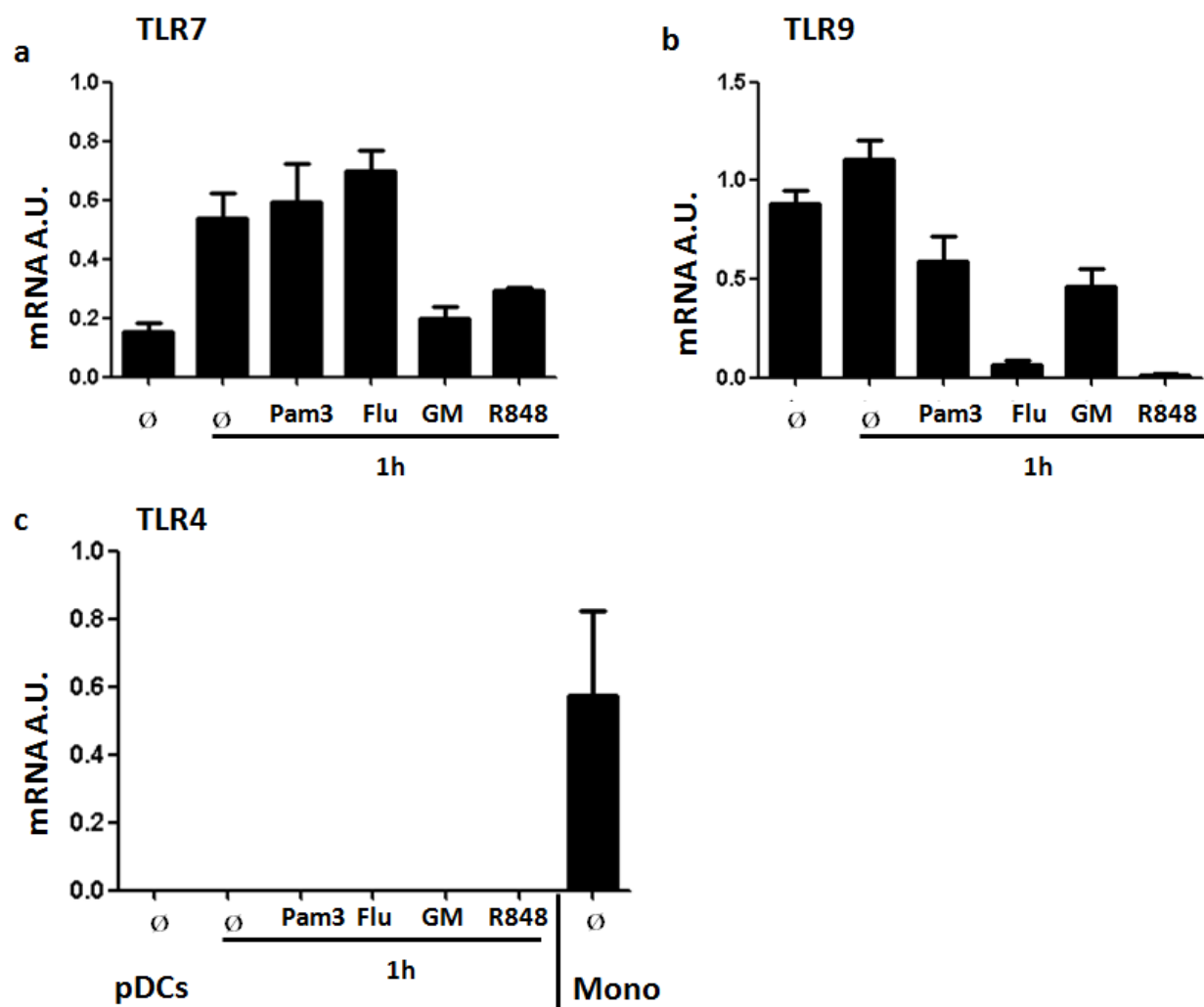


Figure 23: TLRs mRNA expression change

A-C. Real-time quantitative RT-PCR of total mRNA from sorted human pDCs (and after 1 hour activation) and monocytes. pDCs were treated with medium (Ø), PAM3CKS4 (Pam3), influenza virus (flu), GM-CSF (GM), R848. Results were normalized to three housekeeping genes. Results include 5 donors. **A.** TLR7 mRNA expression **B.** TLR9 mRNA expression. **C.** TLR4 mRNA expression. $p < 0.05$; $**p < 0.01$; $***p < 0.001$ (Wilcoxon test)

pDCs were stimulated with increasing doses of PAM3CSK4, to show that pDCs' response to bacterial lipoproteins is dose dependent. We noticed that pDCs respond to the stimulus even at low doses, a result that is in accord with the basal level expression of TLR2.

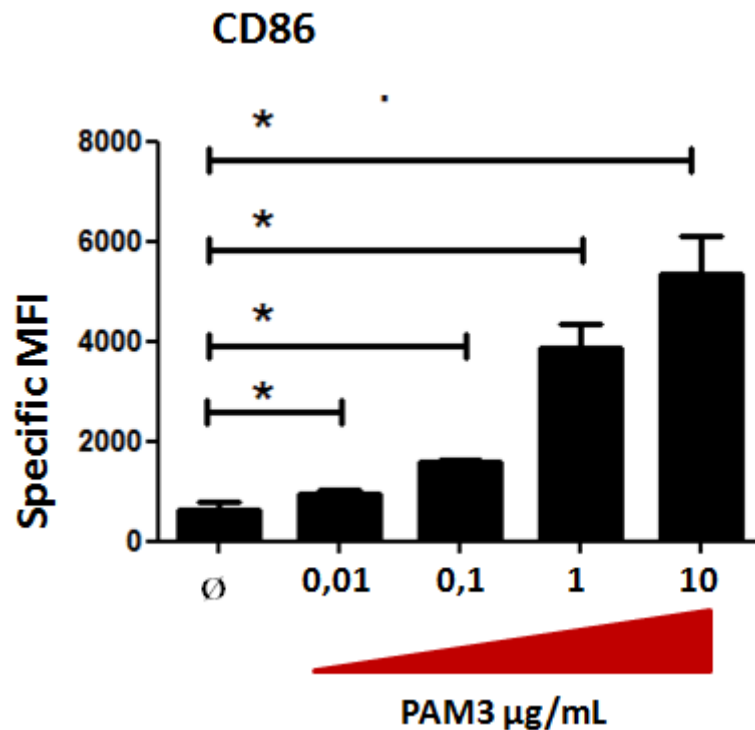


Figure 24: Bacterial lipoproteins activate pDCs in a dose dependent manner

A. Surface expression of co-stimulatory molecules (CD86) in pDCs after 24 hours treatment. pDCs were treated with medium (\emptyset), 4 increasing doses of PAM3CSK4 (PAM3). Results include 7 independent donors. $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Wilcoxon test)

DCs activation also passes through a phenotypical change of the cells. DCs were described for the first time in accordance with their morphology, which differentiates them as granulocytes, lymphocytes and mononuclear phagocytes. pDCs lose their typical round shape with maturation, then acquiring a stellate morphology due to the presence of pseudopods of different lengths.

pDCs, activated by bacterial lipoproteins show phenotypical changes comparable, to an extent, to the influenza virus.

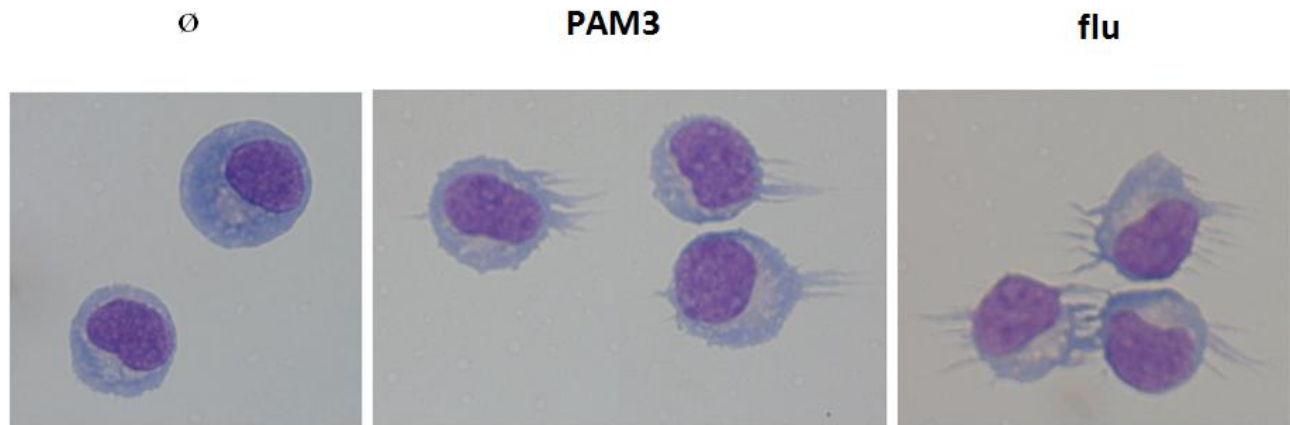


Figure 25: Bacterial lipoproteins treated pDCs undergo phenotypic maturation

A. 24-hour-stimulated pDC cytospin. pDCs were treated with medium (Ø), PAM3CKS4 (Pam3), influenza virus (flu).

Granzyme B is normally considered to be produced by CD8 and NK cells. In particular conditions, some innate cells are able to secrete them. Unstimulated pDCs accumulate in the cytoplasm GrB and are secreted at low levels. The administration of IL-3 and other cytokines, such as GM-CSF and IL-21, to pDCs leads to increased intracellular level secretion. TLR7/TLR9 stimulation, conversely, down-regulates GrB pDCs production at both levels. Despite bacterial lipoproteins signal in the pDCs through TLRs, PAM3CSK4 treatment induces an intracellular, secreted GrB increase.

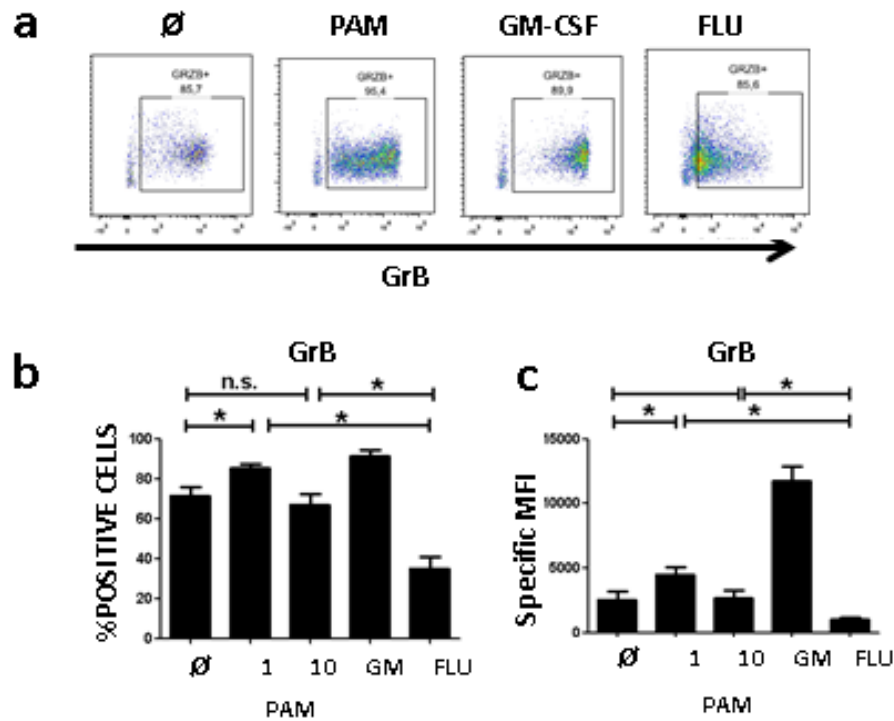


Figure 26: Bacterial lipoprotein-treated pDCs express intracellular granzyme B

A-C. Intracellular FACS was performed after pDCs had been cultured for 24 hours with medium (ø), PAM3CKS4 (Pam3), influenza virus (flu), GM-CSF (GM). **B.** Percentage of positive cells for Granzyme B (GrB). **C.** Specific MFI of GrB. Results include the mean of 7 independent donors. $p < 0.05$; $**p < 0.01$; $***p < 0.001$ (Wilcoxon test).

Considering pDCs express NOD receptors capable of sensing bacterial cell-wall peptidoglycan, we tested NOD receptor for activity in pDCs. We did not notice up-regulation of co-stimulatory molecules (FIG 7), nor cytokine production (data not shown) when pDCs were treated with a NOD ligand. NOD receptors are localized in the cytoplasm and pDCs are poorly engulfing bacteria, which could explain their negligible activation.

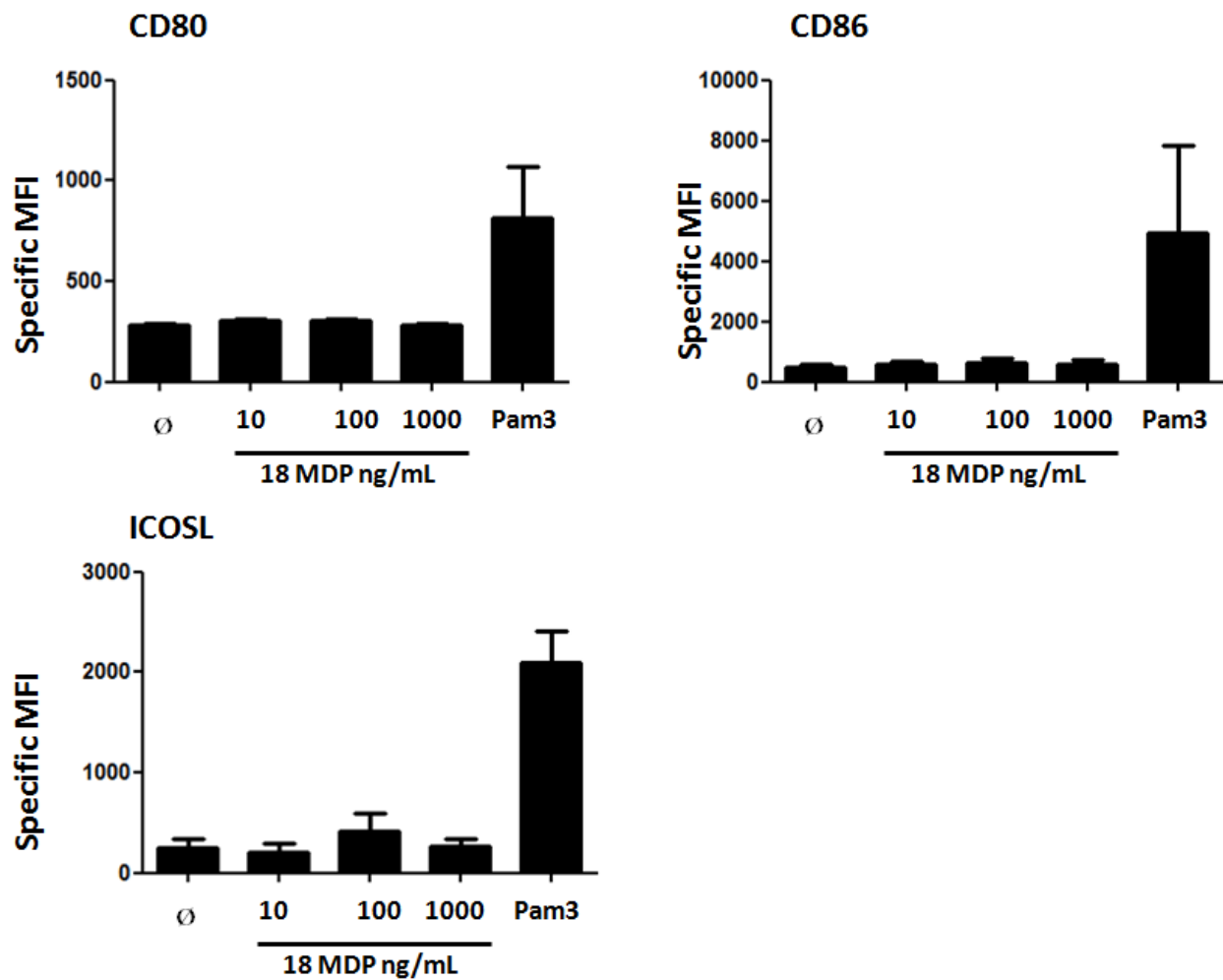


Figure 27: pDCs do not respond to NOD ligand activation

Surface expression of co-stimulatory molecules CD80 (upper-left panel), CD86 (upper-right panel), ICOSL (bottom panel) in pDCs after a 24-hour treatment. pDCs were treated with medium (Ø), PAM3CKS4 (Pam3) and four different doses of 18-MDP (upper-left panel). Results include 4 independent donors.

A real-time quantitative RT-PCR was performed to evaluate the TLRs mRNA expression in tonsil pDCs. The tonsil pDCs present a similar TLRs expression pattern to blood pDCs: high levels of TLR7 and TLR9, comparable expression of TLR1, TLR6 and TLR10.. Moreover, tonsil pDCs express basal levels of TLR2.

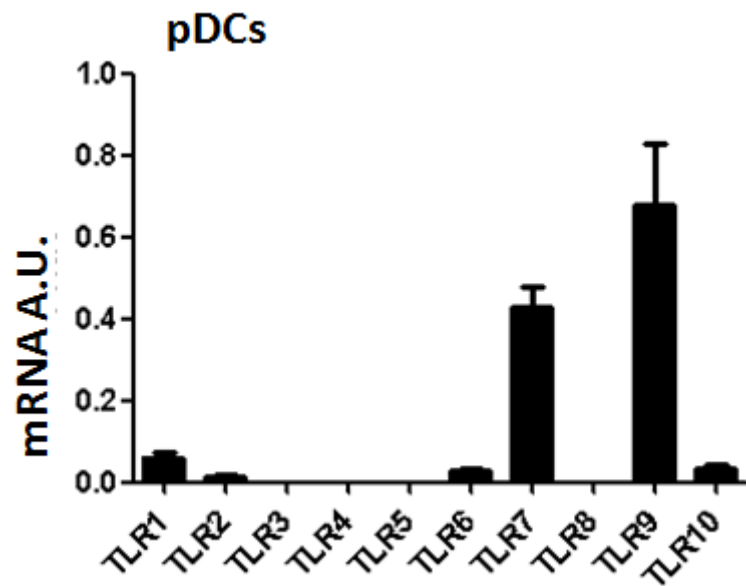


Figure 28: TLRs expression from tonsil pDCs

Real-time RT-PCR from total mRNA from sorted human tonsil pDCs. Results were normalized to three housekeeping genes. Results include 5 independent donors.

DISCUSSION AND PERSPECTIVES

8.1 Antefact

During the introduction I presented the complexity of the immune system. Multi-drug resistance bacteria strains are an increasingly worsening threat and new antibiotics are not yet under way. The evolutionary force of cohabitation between bacteria and mankind has led the former to adapt to our immune system and develop strategies to reverse our weapons to their advantage. a better understanding of the bacterial immune evasion mechanisms can lead us to help the immune system fight them.

8.2 TLR2 and TLR1 expression in pDCs

pDCs are capable of sensing bacteria and respond to them with cytokine secretion and up-regulation of co-stimulatory molecules. If this is an accepted fact, our knowledge about how pDCs sense bacteria and the relevance of their response is still far from a comprehensive view. The important role of TLR2 and TLR1 in the sensing of GRAM+ bacteria and how their relationship affect the immune response outcome was described in the Introduction.

In 2001, Kadowaki stated that pDCs express TLR7 and TLR9 only, among the TLRs family. For a long time, this statement was viewed a “gravestone” for the inquiry on the ability of pDCs to express other TLRs. In 2014, it was reported that mice spleen pDCs express TLR2. pDCs activation through TLR2 was important for driving an anti-inflammatory response to gut commensal antigens. Briefly, pDCs were inducing IL-10 production by CD4 T-cells and dampening the inflammation in an IBD model. In spite of this interesting report, mice pDCs present several differences from their human counterparts, including the fact that they express TLR4, absent from human pDCs. Successive literature referred to the Kadowaki article to cite the missing expression of TLR2/1 from pDCs. Our results showed that steady-state pDCs express TLR1, TLR6 and TLR10 at the mRNA level, in addition to the canonical TLR7 and TLR9. The expression of TLR1 is definitely comparable to the myeloid CD11C+ DCs (all the available literature concords on that myeloid DCs express high levels of TLR1). Moreover, we noticed a small steady-state expression of TLR2, at the mRNA level, in pDCs. This expression is increased following pDCs activation with Pam3CSK4 (a structural analog of

the triacylated lipopeptide, a synthetic chemical compound mimicking the GRAM+ bacterial ligand). pDCs TLR2 expression is lower than the CD11C+ myeloid DCs, although it seems comparable after stimulation. I have confirmed the pDCs TLR2/1 expression at the protein level, showing pDCs are expressing both TLRs, at a low level, on the surface. [²⁹³, ²⁹⁴]

TLRs expression can be modified by the environment; I presented some examples where cytokines are inducing up-regulation or down-regulation of their expression. Our results show that pDCs TLR7 and TLR9 mRNA expression is modified by the stimuli. PAM3CSK4 does not affect TLR7 mRNA expression, while it reduces the expression of TLR9. The influenza virus increases TLR7 expression, while it abrogates the TLR9. R848, a TLR7/8 synthetic ligand, profoundly affects the expression of both TLRs. The influenza virus also activates TLR7 (while also engaging other receptors) and exerts a different effect on TLR7 expression in comparison to R848. This difference is another proof of the system's complexity. pDCs do not express TLR4 and their stimulation does not induce TLR4 mRNA expression. These results show that the TLRs mRNA expression can be modified by the environment but is not an un-regulated variation: it changes according to the circumstances. These differences might rely on the promoter's state; CHIP-seq analysis could unveil the mechanism behind the possible induction of TLR2 but not of TLR4. It has been shown that decreased methylation of the TLR2 promoter results in an up-regulation of TLR2 expression in cystic fibrosis patients, leading to an enhanced pro-inflammatory response. TLRs expression levels were also affected by histone deacetylase complexes (HDAC) inhibitors. However, these changes were not unidirectional, with the expression of some TLRs increasing and that of others decreasing, which suggests a complex regulation yet to be analysed. [²⁹⁵, ²⁹⁶]

It is interesting that the up-regulation of the TLR2 expression happens in the presence of the ligand. pDCs in steady state express TLR1, TLR6 and TLR10 at the mRNA level. All these receptors can heterodimerize with TLR2. These data suggest that TLR2 expression is more strictly regulated and is expressed only in case of necessity. TLR2 can also heterodimerize with other proteins that pDCs do not express (for instance CD14, for which pDCs are negative). TLR2 SNPs, as described

before, are involved in different autoimmunity processes, and this could be one of the reasons for a stricter regulation. Interestingly, it was reported that in multiple sclerosis there are two subsets of pDCs, differentiated from among the other markers by their TLR2 expression. TLR2 expression in pDCs seems more activated in this context and correlating with the pathology. An imbalance of TLR expression on the pDCs can be detrimental and play a role in autoimmunity, as shown by the role played by TLR7 and TLR9 in different diseases. This could be also true for TLR2 and TLR1. This point will be further discussed in the following paragraphs. [²⁹⁷]

8.3 pDCs respond functionally to bacterial lipoprotein.

We further investigated pDCs about their functional capability of sensing bacterial lipoproteins. pDCs were stimulated with different activators (e.g., influenza virus, GM-CSF) and with LPS. It is reported in the literature that pDCs do not express TLR4 and do not respond to LPS. The results showed that pDCs are capable of responding to Pam3CSK4 with co-stimulatory molecules expression and cytokine production. The extent of the response is comparable to the one induced by flu and GM-CSF (higher than GM-CSF for different markers). pDCs are fully capable of responding to the bacterial lipoproteins deriving from GRAM+ bacteria. The different cytokines and co-stimulatory molecules have all roles in T cells priming. Our results show that pDCs are up-regulating the MHC-II complex on the surface, suggesting their capacity to present the antigen to the CD4+ T-cells. CD80, CD86, CD40, CD83 are all involved in the T-cells polarization. Moreover, pDCs up-regulate the expression of ICOSL, which is a key factor to induce IL-10 secretion and proliferation in T cells. Interestingly, pDCs are capable of producing the serine protease Granzyme B (GrB), which can be important to further shape the immune response. They express constitutively in the active form; experiments with the erythro-leukemic cell line K562 showed the pDCs cytotoxic capacity. Furthermore, Grb pDCs production yields T-cell proliferation suppression in a perforin independent manner. Influenza virus treated pDCs (and, in general, other TLRs stimulated pDCs) down-regulate the intracellular GrB production, conversely cytokine (IL-3, IL-21, GM-CSF) stimulate pDCs GrB up-regulation. PAM3CSK4 stimulated pDCs up-regulate intracellular

production of GrB in comparison with medium. It has been observed that pDCs in TB patient lymph nodes present Granzyme B in the cytoplasm. [²⁹⁸, ²⁹⁹, ³⁰⁰, ³⁰¹]

Dendritic cells undergo phenotypically maturation following activation, likewise PAMPs stimulation induces surface modification with the characteristic plasma membrane extroflexions, called dendrits. Bacterial lipoproteins induce these changes in the pDCs shape in a similar extent to influenza virus. The absence of stimulation does not lead to these characteristic shape modifications.

Interesting, pDCs are capable of responding to GRAM- bacteria, but this is not mediated by TLR4 activation. pDCs express TLR7 and TLR9 in endosomal compartments, these receptors can sense ssRNA or dsRNA when the bacteria are engulfed. pDCs lack TLR4 expression and they are not capable of responding to bacteria through this mechanism. Despite having other receptors on the surface that can be implied in the recognition of GRAM- bacteria, these are not part of the TLRs family and they activate different pathways. Instead, GRAM + bacteria activate TLR2/1 on the pDCs surface and this difference could potentially have an interesting meaning. These results suggest pDCs can sense, through TLR7 and TLR9, GRAM+ and GRAM- ssRNA or dsRNA. However, only GRAM+ have the possibility to activate surface TLRs before the engulfment. This allows the cells to receive complementary patterns of information leading them to differentiate the two kinds of pathogens.

For our experiments, pDCs isolated from blood were used. Blood pDCs can be recruited at the site of the infection, but it is very unlikely that blood pDCs enter in contact with the bacteria. We tested if pDCs from tonsils were capable of responding to bacterial lipoproteins. Tonsil is a much more physiological environment; pDCs are quotidianly in contact with GRAM+. The respiratory tract is the entrance door for different pathogens as *M. Tuberculosis* and *S. Aureus*, and pDCs are normally present in the human tonsils, suggesting a possible contact in case of infection. Tonsil pDCs up-regulate co-stimulatory molecules in response to Pam3CSK4 in a similar way to their the

blood counterparts. Moreover, pDCs TLRs mRNA expression is similar to blood pDCs: they express TLR1 at steady state and barely TLR2. This data supports the idea that pDCs are capable of responding to bacterial lipoproteins in the infection tissues after their recruitment.

8.4 pDCs sense GRAM+ bacteria through the TLR2/1 pathway.

It is still controversial how pDCs sense GRAM+ bacteria. Since these are poorly phagocytic, the TLR7/9 contribution could be more theoretical than practical. The TLR2/1 pathway is a good candidate, as these two receptors are expressed on the surface and ready to enter into contact with the microbe when in its presence. On the other hand, bacterial engulfment is not necessary for signaling and, therefore, for pDCs activation. It has already been reported that pDCs respond to *S. Aureus*, but whether pDCs can be activated by *M. Tuberculosis* is still controversial. pDCs are capable of responding to all these three bacteria and, more interestingly, they are able to secrete Type I IFN in response to them. As previously reported in this manuscript, Type I IFN has a controversial role. It is generally accepted that, Type I IFN is implicated in the pathogenesis of *M. Tuberculosis* infection, and the source of this cytokine is still debated. pDCs are able to secrete a high amount of type I IFN in response to these GRAM+ pathogens, and this can affect the disease. Moreover, as previously described in this manuscript, pDCs are recruited during tuberculin skin tests, and are found in the lymph nodes of patients with TB. Our results reinforce the hypothesis that pDCs can be implicated in the GRAM+ infection and explain how pDCs sense them.

Furthermore, we tested pDC sensing of the GRAM+ bacteria for mediation by the TLR2/1 pathway. We used CU-CPT22, a competitive antagonist of PAM3CSK4. After treatment with the antagonist, the co-stimulatory expression induced by the GRAM+ bacteria appeared to be dramatically affected. Likewise, there was an almost complete inhibition of Type I IFN production. On one hand, these data suggest that pDCs recognize GRAM+ bacteria mainly through the TLR2/1 pathway. On the other hand, the data reinforce the importance of the regulation of TLR2 expression in pDCs. Considering that GRAM+ bacteria sensing is mediated by the TLR2/1 pathway,

and the fact that TLR2 can recognize different endogenous ligands, regulating its expression in presence of the ligand could amount to an autoimmunity prevention system. [³⁰²]

pDCs are professional APCs. They are able to prime naïve CD4⁺ T cells and thus shape the final response. We stimulated the pDCs with different GRAM⁺ bacteria and co-cultured them with T cells for 6 days. GRAM⁺ bacteria treated pDCs are capable of inducing proliferation and expansion in the naïve CD4⁺ T cells. These primed T cells are capable of producing cytokines: in fact, we observed a mixed TH subset phenotype. pDCs are able to induce a number of broad polarization, of which the predominant one is the result of the environment where pDCs are primed.

Notwithstanding the efforts to develop a protective vaccine against *S. aureus* remains an extremely tough challenge. Numerous vaccine trials directed towards different toxins and immune evasions factors have failed to pass the test of usefulness. Lipoproteins in GRAM⁺ bacteria represent a consistent part of the proteome and exert a broad range of functions; besides, they also play a role in the disease. In the last few years, lipoproteins have started to be considered promising candidates for a vaccine. *S. aureus* strains defective in lipidation of the pro-LPP (Δlgt) are particularly affected in their virulence (in fact, they are severely impaired in their uptake of iron, among other processes). Some LPPs are expressed in the early phase of the infection cycle, and a murine model vaccine looks rather promising. Moreover, some of these vaccines have triggered a protective immunity boost when used in combination with TLR7 ligands. A better understanding of the pDCs' role in bacterial immune responses can contribute to vaccine development. [³⁰³]

TLR2 and TLR1 can recognize PAMPs from fungi: in fact, β -glucans from different fungi species can be recognized by TLR2. TLR2 also interacts with phospholipo-mannans (PLMs), linear β -1,2-oligomannoside structures that are unique to *C. albicans*. [³⁰⁴, ³⁰⁵] TLR2 also recognizes some unknown ligands from the conidia and hyphae forms of *A. fumigatus*. TLR2/1 and TLR2/6 are receptors for the glucuronoxylomannan (GXM) component of the *C. neoformans*' cell wall.

Contrary to what happens in mice, in humans TLR2/1 but not TLR2/6 recognize different ligands in *A. fumigatus*. [³⁰⁶] In the *A. fumigatus* infections, TLR2 impairment reduces neutrophils recruitment and killing. TLR1 SNPs are associated with higher susceptibility to candidemia. Patients with TLR1 SNPs show a reduced cytokine release from blood monocytes. Interestingly, patients receiving transplanted organs from donors with TLR1 polymorphism are more prone to *Aspergillus* infection. In fungal infection the role of Type I interferon is controversial, apparently helping the development of protective immunity or conversely participating in progression of the disease. The role of pDCs in fungal infection is poorly evaluated. It would be potentially interesting to assess their impact, as well as that of the expression of TLRs and pDCs in this frame. [³⁰⁷,³⁰⁸]

On the whole, it would be interesting to further investigate the role of pDCs in different GRAM+ bacterial infection, in order to evaluate the presence of pDCs, as well as their TLRs expression and activation state. In several studies, mice were challenged with *M. Tuberculosis*, *S. Aureus* and other GRAM+ bacteria to highlight the pathological role of Type I IFN. In the last years, mouse models with depleted pDCs have been generated (E2-2 mutation, BDCA2-DTR mice). It would definitely be interesting to challenge these mouse models with GRAM+ bacteria and evaluate what changes in their immune response. Notably, pDCs not only produce Type I IFN, but also express different molecules on their surface (and secrete other cytokines) which have an important role in the interaction with other immune cells. In this way, an intriguing perspective would be to challenge mice with impaired pDCs, in order to produce Type I IFN. Moreover, taking advantage that different mouse models defective for TLRs (TLR1, TLR2 KO...) are available, it could be interesting to generate TLR1 KO or TLR2 KO mice with depleted pDCs. Another approach could be to analyze pDCs (activation state, TLRs expression and response to bacteria) from patients with recurrent infections or more susceptible to GRAM+ bacteria.

8.5 Differential role of TLR2 and TLR1 in pDCs response to Pam3CSK4

TLR2 and TLR1 hetero-dimerize together in the presence of the ligand; surprisingly, however, the differential block of either receptor exposes some differences. TLR1 blocking affects co-

stimulatory expression, as CD86, CD80 and ICOSL surface expression is remarkably reduced. Moreover, there is a sensible decrease in pro-inflammatory cytokines secretion (IL-6, TNF- α). TLR2 blocking does not produce changes in this activation. Conversely, TLR2 blocking impacts around 90% of the Type I IFN release. These data show the different roles of the two TLRs in controlling the pDCs' response to bacterial lipoproteins.

A simplistic view of receptor cooperation consists in the idea of "digital response": two receptors cooperate to activate the same signaling pathway. Each co-receptor could also be an inhibitor subunit blocking signal transmission. One example is I κ B, which is bound to NF- κ B. I κ B is the inhibitory subunit. Only after its degradation, the two subunits of NF- κ B (p50 and p65) assemble together and translocate into the nucleus to allow transcription. p50 and p65 are both necessary to inducing a response; the knockout of one of these genes silences the response. In other cases, a different subunit exists with a higher or lower affinity to the ligand (IL-2 receptor, for instance). Even if this simplistic view can be seen as a good approach, it may be more realistic to consider that this system has a more complicated regulation. As TLR2 can cooperate with a broader range of receptors, it seems reductive to suppose that changes in the response do not happen in the case of different heterodimerizations. Indeed, some reports postulate the existence of a different signalization between the heterodimers TLR2/1 and TLR2/6. The strength of the cellular activation after the lipopeptide binding seems to depend on the intracellular domains (ICDs) of the TLR1 and TLR6 co-receptors. The exchange of the TLR1 ICD with the TLR6 ICD reduces cytokine release after lipopeptide stimulation. Moreover, the TLR2's intracellular adaptor TIRAP directly connects TLR2/6 to PI3K after the binding, whereas TLR2/1 depends on a currently unknown adaptor, ultimately leading to differences in phosphatidylinositol-(3,4,5)-trisphosphate (PIP3) production and macrophage polarization. In fact, what causes these differences remains an object for speculation: the above evidence is considered inconclusive, and the whole system needs to be studied in depth. In pDCs lipoproteins stimulation a division of labor can be observed, with TLR1 controlling the activation and TLR2 being responsible for the Type I IFN. Considering the pleiotropic effects exerted by Type I IFN, this might explain the importance of a strict regulation of TLR2's expression by pDCs. Furthermore, considering the fact that TLR1 controls co-stimulatory molecule expression,

pro-inflammatory cytokines expression is likely to have a role in T-cell differentiation. pDCs also express TLR6 and TLR10 which can heterodimerize with TLR2. The role of these TLRs in pDCs should be investigated, as unexpected features and present differences in the pDCs activation could emerge. [³⁰⁹, ³¹⁰, ³¹¹]

8.6 Differential role of TLR2 and TLR1 in adaptive immunity priming

We were interested in verifying whether the pDCs' differential activation was reflected in some changes in naïve CD4⁺ T-cell activation. The TLR1 blocking in pDCs has an impact on T-cell expansion; moreover, there is a reduction in the percentage of cycling cells. Thus, TLR1 activation is important in the proliferation of T cells. We further noticed alterations in the cytokine profile after pDCs activation in the presence of the TLRs' differential blocking. TLR1 blocking in pDCs affects the T-cell secretion of IL-13, IL-9, IL-10 and IL-5. We did not observe differences in IFN- γ and IL-3 production; besides, the reduction in TNF- α secretion was small. The reduction in T-cell proliferation and expansion could be explained by the TLR1's blocking impact on co-stimulatory expression and pro-inflammatory cytokines production. IL-10 reduction could be the effect of a lower ICOSL expression by pDCs, previously observed with TLR1 blocking. Whereas a general decrease in the overall cytokines' pattern could be considered a generally reduced T-cell activation, the missing reduction in the TH1 profile (IFN- γ , IL-3 and TNF- α) secretion suggests a different T-helper differentiation profile. IFN- γ and TNF- α are both fundamental for immunity against intracellular bacteria. As previously mentioned, IFN- γ enhances macrophage microbicidal defense and is crucial to the maturation of phagosomes and autophagy. IFN- γ is necessary but not sufficient for mycobacterial control after infection, while TNF- α also plays an important role in the maintenance of granuloma integrity. The absence of both these cytokines is considered deleterious and associated to the host's failure to control the infection. Conversely, IL-4 and IL-13, associated with a TH2 profile, coincide with the inhibition of the autophagic process and with granuloma impairment in TB patients. Likewise, IL-10 plays a pathogenic role in Tuberculosis. Interestingly, T-cell production of these two cytokines is not affected by TLR1 blocking, while the other cytokines are reduced, suggesting TLR1's pathogenic role in these settings.

Because IL-4, IL-5, and IL-13 are generally regarded as being produced by TH2, whereas IL-10 is generally produced by T-regs, we decided to investigate the induction of these subsets. We performed intracellular staining in T-cells on Day 4 to check the expression of master regulator genes (as previously mentioned, these genes control the gene expression profile associated with a particular subset). We observed a decrease in the percentage of cells expressing GATA3 and FoxP3 (master regulator genes respectively for TH2 and T-reg T cells) in the case of the TLR1 blocking. As expected, we did not notice a sensible decrease in Tbet (master regulator gene for TH1). TLR2 does not affect the priming of these T helper subsets. Interestingly, the TLR1 and TLR2 blocking also reduces the expression of BCL-6 (master regulator genes for Tfh). Similarly, we observed a reduction in the MFI in TLR1 blocking for GATA3 and FoxP3, but not for Tbet. These data confirm the intuition of TH2 and Tregs profile as TLR1-activation dependent. pDCs were found enriched in the TB lymph nodes and TH1/TH2/Tregs balance plays a role in bacterial infection. pDCs are able to sense being activated by the GRAM+ bacteria; moreover, they are capable of inducing CD4+ T-cell polarization. Our results show that T-cells are polarized towards a mixed TH pattern; this could be due to the engagement of both receptors. It is interesting to alternately block the two receptors to highlight their different responsibilities in inducing specific TH subsets. These results collectively support a possible role of pDCs in the immune response to bacteria.

Food allergy currently affects an estimated 15 million Americans, and the incidence of inflammatory bowel disease (IBD) has been growing in the last few decades; for both pathologies the relationship among dendritic cells and commensal bacteria plays a crucial role. Studies conducted on animal models as well as actual patients show that oral administration is generally a tolerogenic route and commensal bacteria participate in the immune-modulatory process. It was found that capsular polysaccharide A (PSA) of the gut commensal *Bacteroides fragilis* has an immune-regulatory potential. PSA can induce Tregs generation and release of IL-10, thereby ameliorating pathological inflammation in the gut and even in more distant tissues. In a 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis mouse model, pDC depletion abrogates PSA-mediated protection. Moreover, in the same model, pre-treated PSA pDCs were capable of conferring protection after adoptive transfer (interestingly, myeloid DCs are unable to confer

protection). PSA is a TLR2 homodimers ligand. Interestingly, however, PAM3CSK4 was unable to induce the same protection in a mouse model.^[312] Our results show that in human blood pDCs TLR1 engagement leads to IL-10 induction and Tregs generation. These conflictual results can be explained by: 1) splenic pDCs and environment factors impact were used on the TLR2 pDCs response (for instance, it is reported that TLR7/9-stimulated splenic pDCs induce a strong Tregs response).^[313] 2) mouse and human pDCs are known to present differences and these might have an impact on activation behavior. The literature suggest that what has an impact in the balance between inflammatory and regulatory response is the presence of B7 family molecules (for instance CD80, CD86) or other pro-inflammatory signals. Type I IFN and ICOSL might be important factors in IL-10 induction. In the absence of other factors, Type I IFN can induce IL-10, while the role of ICOSL in IL-10 T-cell release is well known. PSA activation through TLR2 homodimers induces ICOSL and Type I IFN without B7-family co-stimulatory expression. This could explain a strong tolerogenic response. An in-depth investigation of the intimate relationship among TLR2, TLR1, pDCs and commensal bacteria could lead to harness pDCs to ameliorate IBD.

On other hand, gut microbiota influence the antitumor effects of different chemotherapy agents and immunotherapeutic régimes. For instance, optimal oxaliplatin-based chemotherapy requires the immune system to detect components of the gut microbiota, as mice lacking an intact microbiota or MyD88 suffer from reduced antineoplastic effects. Moreover, treatment of GRAM+ bacteria with antibiotics deeply reduces the cyclophosphamide-mediated therapeutic effect in tumor-bearing mice. In the last few years, immune checkpoint blockers have shown to be among the most successful new treatments (for instance, cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and the programmed cell death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1) axis). In principle, their anti-tumor effect is mediated by reactivating T cells driven to an anergic state by the tumor's microenvironment^[314]. The reactivated T cells actively respond against the tumor's antigens. Anti-CTLA4 antibodies can induce colitis and the formation of intestinal lesions; ICOS+ Tregs exert protection, and their activation is thought to be mediated by pDCs activation. An intriguing study shows that the same mouse strain, coming from two different facilities, displays significant differences in melanoma growth following anti PD-1 treatment. These

differences were attributed to the differential presence of GRAM+ *Bifidobacterium* (associated with enhanced control of the tumor growth, restored CTL response and augmented DCs antigen presentation and activation). These studies suggest that the role of pDCs needs be better understood without forgetting the implication of TLR2/TLR1. [³¹⁵]

These TLRs are also capable of recognizing endogenous ligands, as previously described. Moreover, they are involved in the insurgence of autoimmune diseases. The abundant evidence of the pDCs' involvement in autoimmunity is described in the first chapter of this manuscript. BCL-6 up-regulation in T-cell priming suggests the pDCs' capacity to induce Tfh, and their role in B-cell regulation. Furthermore, as described in the Introduction, pDCs can directly or indirectly interact with B-cells. For instance, Type I IFN plays a pivotal role during the maturation of B cells into plasmablasts. pDCs are capable of inducing Bregs (which exhibit immunosuppressive functions via the secretion of IL-10, TGF- β and IL-35), and different studies linked type I IFNs to an increase in IL-10 B-cell production. [³¹⁶] As previously described, pDCs play a role in SLE, where, after endogenous DNA sensing, they induce the production of auto-antibody. To summarize, pDCs' dysregulated activation and Type I IFN role in SLE are associated with the accumulation of plasma cells, increased autoantibody levels, defective apoptotic cell clearance, and promotion of T-cell-dependent inflammation. [³¹⁷, ³¹⁸, ³¹⁹] In SLE, pDCs are more highly activated (higher levels of co-stimulatory expression and cytokines secretion at steady state and upon stimulation). Higher activation inversely correlates with Breg cell frequency. Different levels of Type I IFN can diminish Breg generation, as well as the state of activation of pDCs or the presence of pro-inflammatory signals in the environment's impact on the ratio of Bregs/plasmablasts. [³²⁰] It has been observed that *S. aureus*-treated pDCs induce IL-10 production from B-cells [³²¹]. Our results show that TLR2 control Type I IFN, while pro-inflammatory cytokines and co-stimulatory molecules are TLR1-mediated. To verify how the TLR2/1 pathway regulates interaction with B-cells could be an intriguing challenge. It is possible that each of the two receptors vehicles a different type of input, as seen with the T cells. Antibody response plays a role in the response to extracellular bacteria, while whether it is dispensable during intracellular bacterial infection is still debated. Cross-talk between pDCs and B cells can play a role in this frame. Notably, some studies presented a link

between mycobacterial infection and SLE, both pathologies presenting a relevant Type I IFN signature [322]. The insurgence of other autoimmune diseases (many of which involving autoantibodies) has been diagnosed following TB infection. Wegener's granulomatosis is associated with bacterial infection, especially with *S. aureus*. As the infection may play a role in the induction of autoimmunity, pDC *S. Aureus* sensing can be involved. [323]

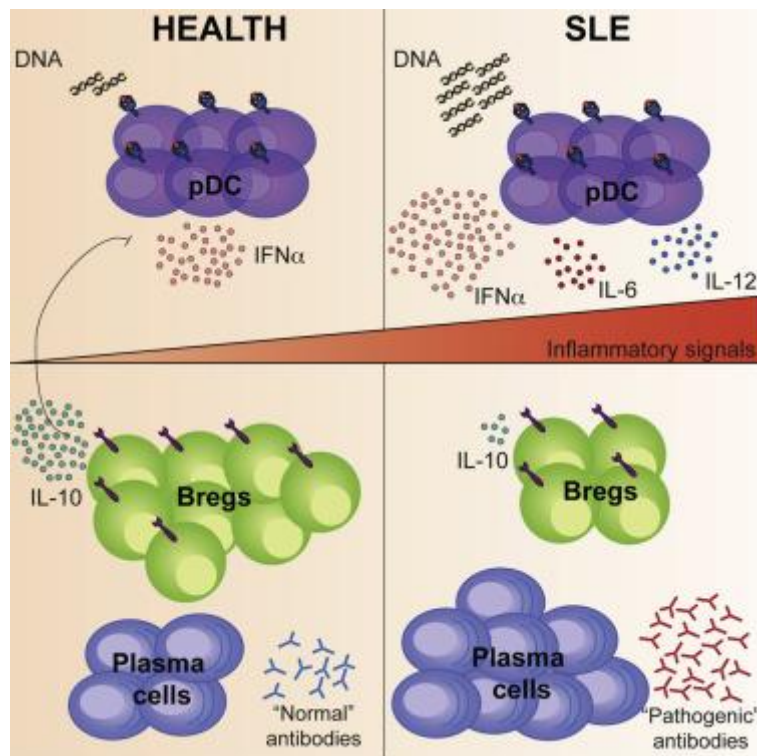


Figure 29: pDCs B-cells pathogenic crosstalk in SLE. Menon et al., 2016.

Germ-free mice show a higher incidence of autoimmune diseases (especially in predisposed models). Pathogen-free mice present controversial results, but the progression of their disease is altered. During different autoimmune diseases there is an imbalance of T cell subsets. In multiple sclerosis effector, Th1 and Th17 cells contribute to tissue inflammation and to the exacerbation of autoimmune pathologies, while Tregs have a protective function. In MS, pDCs are found to drive an immune-regulatory TH9 pathway. Moreover, there one report highlights TLR2 expression by pDCs in patients with a MS active disease. MS patients show reduced IL-9 levels in the

cerebrospinal fluid (CSF) during clinical relapses, but this increases after treatment. Increased IL-9 level in the remission phase was also noticed. In MS, TH9 seems to reduce pathogenic Th17 inflammation and slow down the disease process. Interestingly, our results show that naïve CD4⁺ T cells primed by PAM3-stimulated pDCs produce IL-9; in fact, we have observed its reduction by TLR1 blocking, but not by TLR2 blocking. Furthermore, TLR1 blocking also affects IL-10 production and Tregs polarization. As a future perspective, the TLR1's and TLR2's roles as pDCs in MS should be evaluated. It was reported that germ-free mice show a higher incidence of experimental autoimmune encephalomyelitis (EAE), the murine model for MS, and it has been suggested that microbiota play a protective role. Infection and pDCs could have an impact on the insurgence and/or on the relapses of MS. [³²⁴, ³²⁵]

Myeloid DCs, monocyte-derived DCs (moDC) and macrophages produce IL-12, well known for inducing the TH1 subset. pDCs do not produce IL-12, and how pDCs induce the TH1 and TH2 subsets is still not fully disclosed. TH polarization is influenced by co-stimulatory molecules and a cytokine profile produced by the DCs. Our results show differences in cytokine secretion when one of the two TLR is blocked. Also the co-stimulatory pattern is altered in the differential TLR blocking. These differences are translated into an impact on T-cell polarization. TLR1 blocking affects TH2 development, while TLR2 does not. TLR1 blocking does not affect TH1 polarization. A better knowledge of the dynamics leading to the induction of these different subsets can be important in bacterial infection and autoimmunity. This system can be useful to found new determinants in TH-helper polarization; it could represent a flexible tool for investigating how naïve T-cell are addressed by different subsets. Moreover, in a long-term perspective, alternate blocking of the two TLRs can be helpful towards fine-tuning the pDCs' response of preparing them as a therapeutic weapon.

In future, it would be interesting to perform RNAseq, in order to determine which different gene expression profiles are present in pDCs after the differential blocking of TLR1 and TLR2. pDCs are a node in a complicated network, since they can interact with different cells, as described in the first chapter of this manuscript. These interactions are directly or indirectly mediated by surface molecules or cytokines, respectively. A wider analysis could help to find known and unknown

targets. The differential blocking of the TLRs can put in evidence pDCs' unexpected features and cell partners.

Chapter 4 described the Lorentz force law; these results show a similar behavior: a small change in the initial conditions is amplified during the evolution of the system. Alternate blocking of the two TLRs leads to different patterns at the pDCs level, but these variations amplified in T-cell priming. The generation of different TH profiles leads to a potential alteration in the whole immune response outcome. The potential implications of the dysregulation of these TLRs expression in pDCs could be linked to different pathologies. Factors that alter the balance of these receptors in the cells need consideration in order to evaluate the response of a population to a stimulus. Considering that pDCs can interact with numerous cell types, the pleiotropic effects of Type I IFN, a dysregulation of TLR expression profoundly affects the immune response to pathogen infection. A change on this balance could be also implicated in the autoimmunity insurgence and should be further investigated.

8.7 TLR1 activates a different signaling cascade from TLR2 in pDCs

The differences observed in pDC activation after the differential blocking of the two receptors leave open the question about a mechanistic explanation for this behavior. In the literature, different reports show that there are differences in the signaling pathway engaged during endosomal TLRs activation. The early endosomes TLR7 and TLR9 recruit MyD88 and various components leading to the activation of PI3K and the phosphorylation of AKT, which, in turn phosphorylates IRF7. In pDCs, IRF7 is the master regulator gene controlling Type I IFN. The trafficking of nucleic acids to the late endosomes, instead, leads to the recruitment of different proteins. The signal is still mediated by MyD88, but this leads to activating a MAPK cascade, as well as $\text{Nf-}\kappa\text{B}$. These two pathways lead to, respectively, the induction of co-stimulatory molecule expression and pro-inflammatory cytokine production. TLR2/1 signaling has been studied in cell lines and in myeloid cells but not in pDCs. TLR2/1 activates PI3K, MAPK and $\text{Nf-}\kappa\text{B}$ pathways through MyD88.

Our results show that lipoprotein stimulation in pDCs activates PI3K, MAPK and Nf- κ B pathways. TLR1 blocking reduces the phosphorylation of p38 and p65, showing that MAPK and Nf- κ B are TLR1-dependent. These data are coherent with the previous results, i.e., a decrease in co-stimulatory and pro-inflammatory cytokines. TLR2 blocking leads to a reduction in the phosphorylation of AKT. PI3K is essential to IRF7 nuclear translocation and its Type I IFN gene transcription. Some reports suggest that in inflammatory monocytes and bone marrow-derived macrophages, TLR2 can be internalized and lead to IRF7 activation. Barbalat *et al.* showed that TLR2 activation leads to IRF7 activation in the inflammatory monocytes of mice. TLR2 engagement by virus leads to Type I IFN induction, but this induction was only viral ligand-dependent, not bacterial ligand-dependent. In the mouse model of *Listeria* infection, TLR2 engagement leads to Type I IFN production in a IRF7-dependent way. It has been suggested that TLR2 can be internalized in murine macrophages, while in the endosome it recruits IRF7 and induces Type I IFN. There are no studies on human cells confirming the engagement of IRF7 after TLR2 activation. All previous studies with bacterial lipoproteins suggest that the TLR2/1 complex signals through MAPK and Nf- κ B and, in addition, through IRF7 activation. In pDCs, the two receptors induce different pathways, one leading to co-stimulatory molecule expression and pro-inflammatory cytokine secretion, the other inducing type-I IFN release. These data provide a mechanistic explanation for the phenotype previously observed. Within the frame of TLR7 or TLR9 pDC activation, the spatiotemporal regulation leads to a differential signaling pathway engagement. TLR2 and TLR1 are expressed on the surface, and there is some evidence that they can be internalized and directed to the endosome. [³²⁶] It is possible that the lipoprotein binding to TLR2/1 leads to the internalization and consequential recruitment of IRF7 through Myd88. pDCs might fail to internalize the lipoprotein receptor complex or recruit IRF7 during TLR2 blocking. As previously described, in the absence of a ligand, these TLR receptors float freely on the surface. The ligand binding induces the receptor hetero-dimerization and migration to lipid rafts. During activation, the lipid rafts change in composition and lead to the recruitment of adaptor proteins. The blocking of TLR1 or TLR2 could have an impact on the migration to the lipid rafts. It is possible that interfering with the receptor's migration to lipid rafts does not allow the activation of a signaling pathway. Eventually, TLR blocking can interfere with the recruitment of adaptor

molecules to the lipid rafts. Spatiotemporal regulation of the two TLRs remains an open question and the answer to it could explain the different activation of these signaling pathways by TLR2 and TLR1. Further investigation is also necessary to decipher the physiological role of the engagement of different pathways by the two receptors. [³²⁷, ³²⁸]

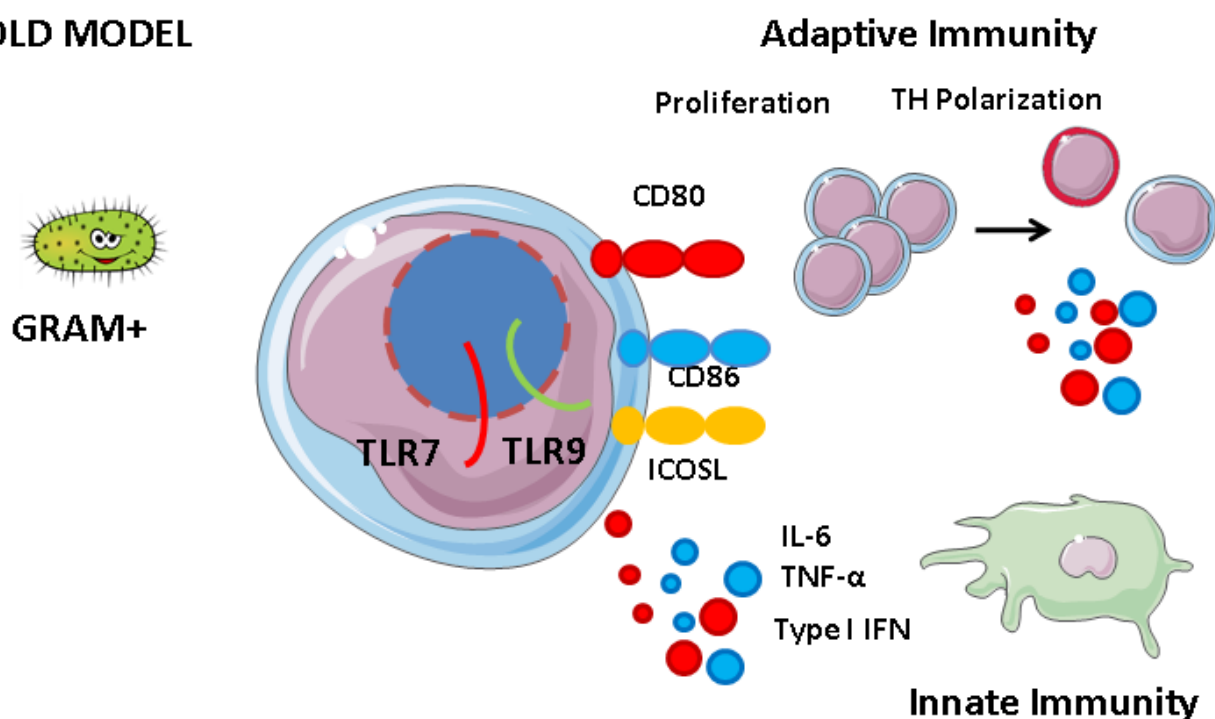
DNA methylation, histones acetylation and deacetylation altering the structure of chromatin are fundamental in the regulation of gene expression, cellular proliferation, cell cycle and differentiation. Alteration in the epigenetic landscape has been linked to tumorigenesis as well as autoimmune diseases. In the last few years, some reports have highlighted the role of these modifications in the resolution of bacterial infection. One study shows that methylation of the heparin-binding hemagglutinin (HBHA) protein was necessary for effective immune defence against *Mycobacterium tuberculosis*. Methylated HBHA was important for the induction of T-cell antigenicity during protective immunity against MTB [³²⁹].

Although the role of DNA methylation and histone modifications in T-cell survival and TH polarization is well known, its relevance in DC activation has also been highlighted. For instance, histone deacetylase complexe (HDAC) inhibitors affect cytokine secretion following TLR3/4 activation. [³³⁰] On the other hand, it has been noticed in mouse macrophages that TLR4 activation with LPS results in the repression of certain HDACs and an increased expression of others. Moreover, LAQ824 (an HDAC inhibitor) treatment to TLR4 activated macrophages and dendritic cells alters their function: dendritic cells are unable to prime TH1, while TH2 priming remains unaffected. [³³¹] In pDC bacterial lipoproteins activation some epigenetic factors can be involved as part of a mechanistic explanation for the specific cytokine secretion dependent on each of the TLRs. TLR1 and TLR2 could differentially activate or repress some HDAC, methyl-transferases (DNMTs) and acetyl-transferases in order to induce the expression of pro-inflammatory cytokines, Type I IFN and co-stimulatory molecules. The blocking of TLR's dysregulated expression can alter this pattern of epigenetic regulators and lead to variation in the immune response. [³³², ³³³]

8.8 Concluding remarks

The old view represents pDCs as responding to GRAM+ bacteria through the sensing of their nucleic acid, mature and prime T-cells. pDCs engulf the bacteria poorly, leaving the question open. Our results show the likelihood of a different model: pDCs can sense GRAM+ through TLR2/1 without the need to engulf the bacteria. This model suggests that the two receptors play different roles, allowing a more finely tuned response. The division of labor between the two TLRs allows the cells to adjust the response to different challenges. Conversely, an up-regulation of one receptor can skew the response to a pathogenic one. These results suggest that there is an adjunctive level of complexity to be considered in the frame of TLRs' activation, which might extend to receptor cooperation. Our data could also help explain conflictual results about TLR2s' role in infective diseases where some studies show their protective role in bacterial infection and others suggest a detrimental impact. TLR2 DC activation needs to be considered in concert with the other expressed co-receptors. The balance between these receptors chooses the final outcome response; small variations in this game can profoundly affect the final results. The environment or genetic factors can modify TLR expression by dendritic cells, leading to the onset of disease. Notably, our work shows that the system is more complex, especially for receptors that hetero-dimerize, as the two TLRs are not redundant but transmit different information in a non-digital response.

OLD MODEL



NEW PROPOSED MODEL

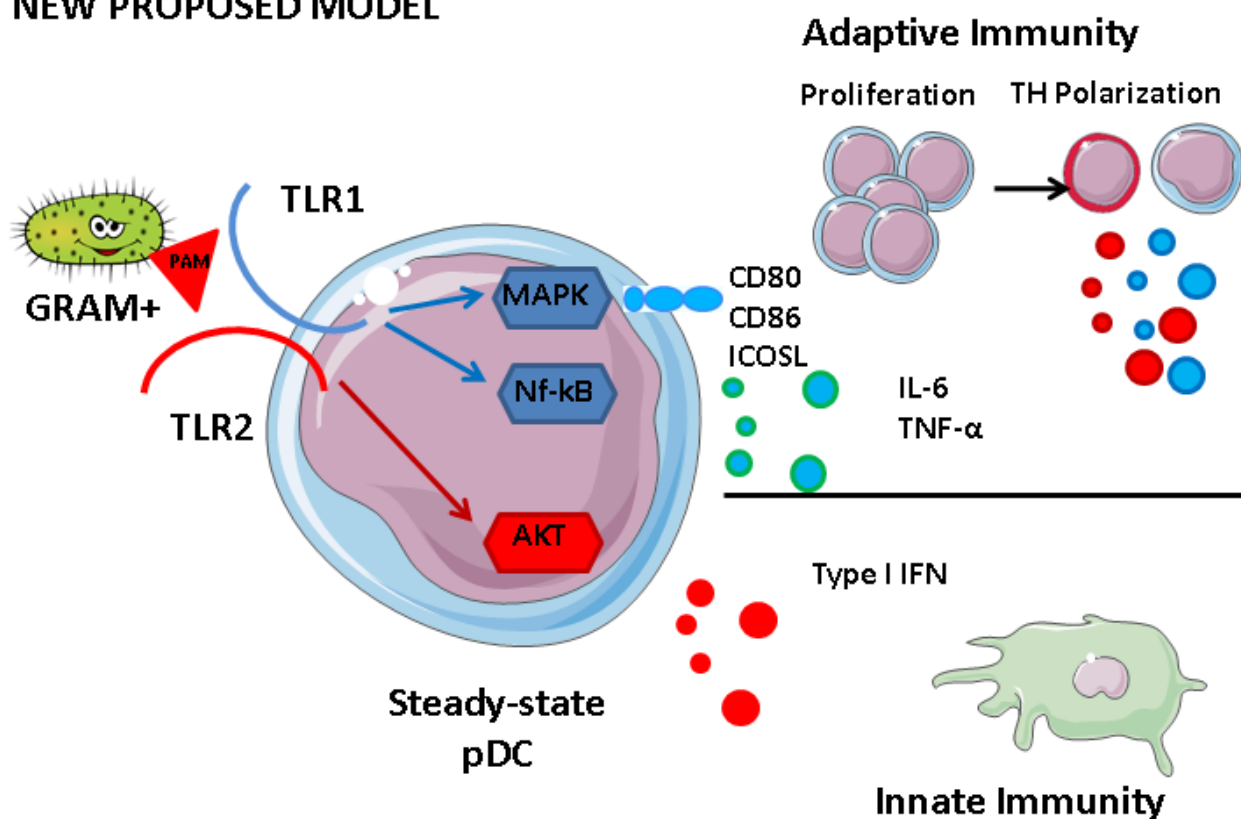


Figure 30: New proposed model of pDC GRAM+ bacteria sensing. Old model of pDCs GRAM+ bacteria sensing is mediated by the endosomal TLRs leading to pDCs activation and maturation. Activated pDCs express co-stimulatory molecules and secreting cytokines in order to prime T-cells and converse with innate immunity. The new model shows pDCs sensing the GRAM+ bacteria through TLR2/1. TLR1 activates MAPK and NF- κ B pathway inducing co-stimulatory molecules and pro-inflammatory cytokines and regulating T-cells proliferation and polarization. TLR2 specific controls Type I IFN secretion to alert innate immunity.

REFERENCES

1. WC Van Voorhis et al., "Human Dendritic Cells. Enrichment and Characterization from Peripheral Blood," *The Journal of Experimental Medicine* 155, no. 4 (April 1, 1982): 1172–87, doi:10.1084/jem.155.4.1172.
2. Loems Ziegler-Heitbrock et al., "Nomenclature of Monocytes and Dendritic Cells in Blood," *Blood* 116, no. 16 (October 21, 2010): e74–80, doi:10.1182/blood-2010-02-258558.
3. Motonari Kondo et al., "BIOLOGY OF HEMATOPOIETIC STEM CELLS AND PROGENITORS: Implications for Clinical Application," *Annual Review of Immunology* 21, no. 1 (2003): 759–806, doi:10.1146/annurev.immunol.21.120601.141007.
4. Markus G. Manz et al., "Dendritic Cell Potentials of Early Lymphoid and Myeloid Progenitors," *Blood* 97, no. 11 (June 1, 2001): 3333–41, doi:10.1182/blood.V97.11.3333.
5. Lionel Franz Poulin et al., "Characterization of Human DNGR-1 + BDCA3 + Leukocytes as Putative Equivalents of Mouse CD8 α + Dendritic Cells," *The Journal of Experimental Medicine* 207, no. 6 (June 7, 2010): 1261–71, doi:10.1084/jem.20092618.
6. Olivier P. Joffre et al., "Cross-Presentation by Dendritic Cells," *Nature Reviews Immunology* 12, no. 8 (August 2012): 557–69, doi:10.1038/nri3254.
7. Jacques Banchereau et al., "Immunobiology of Dendritic Cells," *Annual Review of Immunology* 18, no. 1 (2000): 767–811, doi:10.1146/annurev.immunol.18.1.767.
8. Nermina Malanovic and Karl Lohner, "Antimicrobial Peptides Targeting Gram-Positive Bacteria," *Pharmaceuticals* 9, no. 4 (September 20, 2016): 59, doi:10.3390/ph9030059.
9. Carine Asselin-Paturel et al., "Mouse Type I IFN-Producing Cells Are Immature APCs with Plasmacytoid Morphology," *Nature Immunology* 2, no. 12 (December 2001): 1144–50, doi:10.1038/ni736.
10. Michel Gilliet et al., "The Development of Murine Plasmacytoid Dendritic Cell Precursors Is Differentially Regulated by FLT3-Ligand and Granulocyte/Macrophage Colony-Stimulating Factor," *The Journal of Experimental Medicine* 195, no. 7 (April 1, 2002): 953–58, doi:10.1084/jem.20020045.

11. Wei Chen et al., "Thrombopoietin Cooperates with FLT3-Ligand in the Generation of Plasmacytoid Dendritic Cell Precursors from Human Hematopoietic Progenitors," *Blood* 103, no. 7 (April 1, 2004): 2547–53, doi:10.1182/blood-2003-09-3058.
12. Proietto et al., "The Equivalents of Human Blood and Spleen Dendritic Cell Subtypes Can Be Generated in Vitro from Human CD34+ Stem Cells in the Presence of Fms-like Tyrosine Kinase 3 Ligand and Thrombopoietin," *Cellular & Molecular Immunology* 9, no. 6 (November 2012): 446–54, doi:10.1038/cmi.2012.48.
13. Ben Fancke et al., "M-CSF: A Novel Plasmacytoid and Conventional Dendritic Cell Poietin," *Blood* 111, no. 1 (January 1, 2008): 150–59, doi:10.1182/blood-2007-05-089292.
14. Tobias K. Vogt et al., "Novel Function for Interleukin-7 in Dendritic Cell Development," *Blood* 113, no. 17 (April 23, 2009): 3961–68, doi:10.1182/blood-2008-08-176321.
15. Hideki Tsujimura, Tomohiko Tamura, and Keiko Ozato, "Cutting Edge: IFN Consensus Sequence Binding Protein/IFN Regulatory Factor 8 Drives the Development of Type I IFN-Producing Plasmacytoid Dendritic Cells," *The Journal of Immunology* 170, no. 3 (February 1, 2003): 1131–35, doi:10.4049/jimmunol.170.3.1131.
16. Maho Nagasawa et al., "Development of Human Plasmacytoid Dendritic Cells Depends on the Combined Action of the Basic Helix-Loop-Helix Factor E2-2 and the Ets Factor Spi-B," *European Journal of Immunology* 38, no. 9 (September 2008): 2389–2400, doi:10.1002/eji.200838470.
17. Hiya S. Ghosh et al., "Continuous Expression of the Transcription Factor E2-2 Maintains the Cell Fate of Mature Plasmacytoid Dendritic Cells," *Immunity* 33, no. 6 (December 2010): 905–16, doi:10.1016/j.immuni.2010.11.023.
18. Yasmina Laouar et al., "STAT3 Is Required for Flt3L-Dependent Dendritic Cell Differentiation," *Immunity* 19, no. 6 (December 2003): 903–12, doi:10.1016/S1074-7613(03)00332-7.
19. Babacar Cisse et al., "Transcription Factor E2-2 Is an Essential and Specific Regulator of Plasmacytoid Dendritic Cell Development," *Cell* 135, no. 1 (October 2008): 37–48, doi:10.1016/j.cell.2008.09.016.

20. Lynn Corcoran et al., "The Lymphoid Past of Mouse Plasmacytoid Cells and Thymic Dendritic Cells," *The Journal of Immunology* 170, no. 10 (May 15, 2003): 4926–32, doi:10.4049/jimmunol.170.10.4926.
21. Shalin H. Naik et al., "Development of Plasmacytoid and Conventional Dendritic Cell Subtypes from Single Precursor Cells Derived in Vitro and in Vivo," *Nature Immunology* 8, no. 11 (November 2007): 1217–26, doi:10.1038/ni1522.
22. Nobuyuki Onai et al., "A Clonogenic Progenitor with Prominent Plasmacytoid Dendritic Cell Developmental Potential," *Immunity* 38, no. 5 (May 2013): 943–57, doi:10.1016/j.immuni.2013.04.006.
23. Boris Reizis et al., "Plasmacytoid Dendritic Cells: Recent Progress and Open Questions," *Annual Review of Immunology* 29, no. 1 (2011): 163–83, doi:10.1146/annurev-immunol-031210-101345.
24. Giuseppe Penna et al., "Differential Migration Behavior and Chemokine Production by Myeloid and Plasmacytoid Dendritic Cells," *Human Immunology* 63, no. 12 (December 2002): 1164–71, doi:10.1016/S0198-8859(02)00755-3.
25. Marina Cella et al., "Plasmacytoid Monocytes Migrate to Inflamed Lymph Nodes and Produce Large Amounts of Type I Interferon," *Nature Medicine* 5, no. 8 (August 1999): 919–23, doi:10.1038/11360.
26. Hiroyuki Yoneyama et al., "Evidence for Recruitment of Plasmacytoid Dendritic Cell Precursors to Inflamed Lymph Nodes through High Endothelial Venules," *International Immunology* 16, no. 7 (July 1, 2004): 915–28, doi:10.1093/intimm/dxh093.
27. Giuseppe Penna et al., "Cutting Edge: Selective Usage of Chemokine Receptors by Plasmacytoid Dendritic Cells," *The Journal of Immunology* 167, no. 4 (August 15, 2001): 1862–66, doi:10.4049/jimmunol.167.4.1862.
28. Jietang Mai et al., "An Evolving New Paradigm: Endothelial Cells – Conditional Innate Immune Cells," *Journal of Hematology & Oncology* 6, no. 1 (2013): 61, doi:10.1186/1756-8722-6-61.

29. William Vermi et al., "Role of ChemR23 in Directing the Migration of Myeloid and Plasmacytoid Dendritic Cells to Lymphoid Organs and Inflamed Skin," *The Journal of Experimental Medicine* 201, no. 4 (February 21, 2005): 509–15, doi:10.1084/jem.20041310.
30. Jurjen Tel et al., "Human Plasmacytoid Dendritic Cells Phagocytose, Process, and Present Exogenous Particulate Antigen," *The Journal of Immunology* 184, no. 8 (April 15, 2010): 4276–83, doi:10.4049/jimmunol.0903286.
31. Jean-Baptiste Guillerme et al., "Measles Virus Vaccine–Infected Tumor Cells Induce Tumor Antigen Cross-Presentation by Human Plasmacytoid Dendritic Cells," *American Association for Cancer Research* 19, no. 5 (March 1, 2013): 1147–58, doi:10.1158/1078-0432.CCR-12-2733.
32. Norimitsu Kadowaki et al., "Natural Interferon α/β –Producing Cells Link Innate and Adaptive Immunity," *The Journal of Experimental Medicine* 192, no. 2 (July 17, 2000): 219–26, doi:10.1084/jem.192.2.219.
33. Marie-Clotilde Rissoan et al., "Reciprocal Control of T Helper Cell and Dendritic Cell Differentiation," *Science* 283, no. 5405 (February 19, 1999): 1183–86, doi:10.1126/science.283.5405.1183.
34. Tomoki Ito et al., "Plasmacytoid Dendritic Cells Regulate Th Cell Responses through OX40 Ligand and Type I IFNs," *The Journal of Immunology* 172, no. 7 (April 1, 2004): 4253–59, doi:10.4049/jimmunol.172.7.4253.
35. Marina Cella et al., "Plasmacytoid Dendritic Cells Activated by Influenza Virus and CD40L Drive a Potent TH1 Polarization," *Nature Immunology* 1, no. 4 (October 2000): 305–10, doi:10.1038/79747.
36. Tomoki Ito et al., "Plasmacytoid Dendritic Cells Prime IL-10–producing T Regulatory Cells by Inducible Costimulator Ligand," *The Journal of Experimental Medicine* 204, no. 1 (January 22, 2007): 105–15, doi:10.1084/jem.20061660.
37. Makoto Ogata et al., "Plasmacytoid Dendritic Cells Have a Cytokine-Producing Capacity to Enhance ICOS Ligand-Mediated IL-10 Production during T-Cell Priming," *International Immunology* 25, no. 3 (March 1, 2013): 171–82, doi:10.1093/intimm/dxs103.

38. Laurence Chaperot et al., "Virus or TLR Agonists Induce TRAIL-Mediated Cytotoxic Activity of Plasmacytoid Dendritic Cells," *The Journal of Immunology* 176, no. 1 (January 1, 2006): 248–55, doi:10.4049/jimmunol.176.1.248.
39. Claire Germain et al., "Induction of Lectin-like Transcript 1 (LLT1) Protein Cell Surface Expression by Pathogens and Interferon- γ Contributes to Modulate Immune Responses," *Journal of Biological Chemistry* 286, no. 44 (November 4, 2011): 37964–75, doi:10.1074/jbc.M111.285312.
40. Isabelle Béatrice Bekeredjian-Ding et al., "Plasmacytoid Dendritic Cells Control TLR7 Sensitivity of Naïve B Cells via Type I IFN," *The Journal of Immunology* 174, no. 7 (April 1, 2005): 4043–50, doi:10.4049/jimmunol.174.7.4043.
41. Joanne Shaw et al., "Plasmacytoid Dendritic Cells Regulate B-Cell Growth and Differentiation via CD70," *Blood* 115, no. 15 (April 15, 2010): 3051–57, doi:10.1182/blood-2009-08-239145.
42. Julien J. Karrich et al., "IL-21–stimulated Human Plasmacytoid Dendritic Cells Secrete Granzyme B, Which Impairs Their Capacity to Induce T-Cell Proliferation," *Blood* 121, no. 16 (April 18, 2013): 3103–11, doi:10.1182/blood-2012-08-452995.
43. Begoña Pérez-Cabezas et al., "TLR-Activated Conventional DCs Promote γ -Secretase-Mediated Conditioning of Plasmacytoid DCs," *Journal of Leukocyte Biology* 92, no. 1 (July 1, 2012): 133–43, doi:10.1189/jlb.0911452.
44. Shino Hanabuchi et al., "Human Plasmacytoid Predendritic Cells Activate NK Cells through Glucocorticoid-Induced Tumor Necrosis Factor Receptor-Ligand (GITRL)," *Blood* 107, no. 9 (May 1, 2006): 3617–23, doi:10.1182/blood-2005-08-3419.
45. Rebekka Wehner et al., "The Bidirectional Crosstalk between Human Dendritic Cells and Natural Killer Cells," *Journal of Innate Immunity* 3, no. 3 (2011): 258–63, doi:10.1159/000323923.
46. Carlos J. Montoya et al., "Activation of Plasmacytoid Dendritic Cells with TLR9 Agonists Initiates Invariant NKT Cell-Mediated Cross-Talk with Myeloid Dendritic Cells," *The Journal of Immunology* 177, no. 2 (July 15, 2006): 1028–39, doi:10.4049/jimmunol.177.2.1028.

47. Isabelle Bekeredjian-Ding et al., "Tumour-Derived Prostaglandin E2 and Transforming Growth Factor- β Synergize to Inhibit Plasmacytoid Dendritic Cell-Derived Interferon- α ," *Immunology* 128, no. 3 (November 1, 2009): 439–50, doi:10.1111/j.1365-2567.2009.03134.x.
48. Aihua Li et al., "IL-8 Directly Enhanced Endothelial Cell Survival, Proliferation, and Matrix Metalloproteinases Production and Regulated Angiogenesis," *The Journal of Immunology* 170, no. 6 (March 15, 2003): 3369–76, doi:10.4049/jimmunol.170.6.3369.
49. Madeleine L. Kalb et al., "TRAIL+ Human Plasmacytoid Dendritic Cells Kill Tumor Cells In Vitro: Mechanisms of Imiquimod- and IFN- α -Mediated Antitumor Reactivity," *The Journal of Immunology* 188, no. 4 (February 15, 2012): 1583–91, doi:10.4049/jimmunol.1102437.
50. Cristina Ghirelli, Raphaël Zollinger, and Vassili Soumelis, "Systematic Cytokine Receptor Profiling Reveals GM-CSF as a Novel TLR-Independent Activator of Human Plasmacytoid Predendritic Cells," *Blood* 115, no. 24 (June 17, 2010): 5037–40, doi:10.1182/blood-2010-01-266932.
51. Cristina Ghirelli et al., "Breast Cancer Cell-Derived GM-CSF Licenses Regulatory Th2 Induction by Plasmacytoid Predendritic Cells in Aggressive Disease Subtypes," *Cancer Research* 75, no. 14 (July 15, 2015): 2775–87, doi:10.1158/0008-5472.CAN-14-2386.
52. Vanja Sisirak et al., "Genetic Evidence for the Role of Plasmacytoid Dendritic Cells in Systemic Lupus Erythematosus," *The Journal of Experimental Medicine* 211, no. 10 (September 22, 2014): 1969–76, doi:10.1084/jem.20132522.
53. Sarah L. Rowland et al., "Early, Transient Depletion of Plasmacytoid Dendritic Cells Ameliorates Autoimmunity in a Lupus Model," *The Journal of Experimental Medicine* 211, no. 10 (September 22, 2014): 1977–91, doi:10.1084/jem.20132620.
54. Laura M. Davison and Trine N. Jørgensen, "Sialic Acid-Binding Immunoglobulin-Type Lectin H-Positive Plasmacytoid Dendritic Cells Drive Spontaneous Lupus-like Disease Development in B6.Nba2 Mice," *Arthritis & Rheumatology* 67, no. 4 (April 1, 2015): 1012–22, doi:10.1002/art.38989.
55. Melissa Swiecki et al., "Plasmacytoid Dendritic Cell Ablation Impacts Early Interferon Responses and Antiviral NK and CD8+ T Cell Accrual," *Immunity* 33, no. 6 (December 2010): 955–66, doi:10.1016/j.immuni.2010.11.020.

56. Roberto Baccala et al., "Anti-IFN- α/β Receptor Antibody Treatment Ameliorates Disease in Lupus-Predisposed Mice," *The Journal of Immunology* 189, no. 12 (December 15, 2012): 5976–84, doi:10.4049/jimmunol.1201477.
57. Tanja Lövgren et al., "Induction of Interferon- α Production in Plasmacytoid Dendritic Cells by Immune Complexes Containing Nucleic Acid Released by Necrotic or Late Apoptotic Cells and Lupus IgG," *Arthritis & Rheumatism* 50, no. 6 (June 2004): 1861–72, doi:10.1002/art.20254.
58. Lynda Bennett et al., "Interferon and Granulopoiesis Signatures in Systemic Lupus Erythematosus Blood," *Journal of Experimental Medicine* 197, no. 6 (March 17, 2003): 711–23, doi:10.1084/jem.20021553.
59. Jane Tian et al., "Toll-like Receptor 9-dependent Activation by DNA-Containing Immune Complexes Is Mediated by HMGB1 and RAGE," *Nature Immunology* 8, no. 5 (May 2007): 487–96, doi:10.1038/ni1457.
60. Paul M. Gallo et al., "Amyloid-DNA Composites of Bacterial Biofilms Stimulate Autoimmunity," *Immunity* 42, no. 6 (June 2015): 1171–84, doi:10.1016/j.immuni.2015.06.002.
61. Jeremy Di Domizio et al., "Nucleic Acid-Containing Amyloid Fibrils Potently Induce Type I Interferon and Stimulate Systemic Autoimmunity," *Proceedings of the National Academy of Sciences* 109, no. 36 (September 4, 2012): 14550–55, doi:10.1073/pnas.1206923109.
62. Mikhail Pashenkov et al., "Two Subsets of Dendritic Cells Are Present in Human Cerebrospinal Fluid," *Brain* 124, no. 3 (March 1, 2001): 480–92, doi:10.1093/brain/124.3.480.
63. Mikhail Pashenkov et al., "Recruitment of Dendritic Cells to the Cerebrospinal Fluid in Bacterial Neuroinfections," *Journal of Neuroimmunology* 122, no. 1–2 (January 2002): 106–16, doi:10.1016/S0165-5728(01)00451-9.
64. Nicholas Schwab et al., "An Imbalance of Two Functionally and Phenotypically Different Subsets of Plasmacytoid Dendritic Cells Characterizes the Dysfunctional Immune Regulation in Multiple Sclerosis," *The Journal of Immunology* 184, no. 9 (May 1, 2010): 5368–74, doi:10.4049/jimmunol.0903662.

65. Frank O. Nestle et al., "Plasmacytoid Predendritic Cells Initiate Psoriasis through Interferon- α Production," *The Journal of Experimental Medicine* 202, no. 1 (July 4, 2005): 135–43, doi:10.1084/jem.20050500.
66. Cristina Albanesi et al., "Chemerin Expression Marks Early Psoriatic Skin Lesions and Correlates with Plasmacytoid Dendritic Cell Recruitment," *The Journal of Experimental Medicine* 206, no. 1 (January 16, 2009): 249–58, doi:10.1084/jem.20080129.
67. Dana Saadeh, Mazen Kurban, and Ossama Abbas, "Update on the Role of Plasmacytoid Dendritic Cells in Inflammatory/autoimmune Skin Diseases," *Experimental Dermatology* 25, no. 6 (June 2016): 415–21, doi:10.1111/exd.12957.
68. Julien Diana et al., "Crosstalk between Neutrophils, B-1a Cells and Plasmacytoid Dendritic Cells Initiates Autoimmune Diabetes," *Nature Medicine* 19, no. 1 (January 2013): 65–73, doi:10.1038/nm.3042.
69. Qing Li et al., "Interferon- α Initiates Type 1 Diabetes in Nonobese Diabetic Mice," *Proceedings of the National Academy of Sciences* 105, no. 34 (August 26, 2008): 12439–44, doi:10.1073/pnas.0806439105.
70. Adam G. Peres and Joaquín Madrenas, "The Broad Landscape of Immune Interactions with *Staphylococcus Aureus*: From Commensalism to Lethal Infections," *Burns* 39, no. 3 (May 2013): 380–88, doi:10.1016/j.burns.2012.12.008.
71. J. C. Lawrence, "Burn Bacteriology during the Last 50 Years," *Burns* 18 (January 1992): S23–29, doi:10.1016/0305-4179(92)90066-4.
72. Henry R. Shinefield and Naomi L. Ruff, "Staphylococcal Infections: A Historical Perspective," *Infectious Disease Clinics of North America, Staphylococcal Infections*, 23, no. 1 (March 2009): 1–15, doi:10.1016/j.idc.2008.10.007.
73. Heiman FL Wertheim et al., "Risk and Outcome of Nosocomial *Staphylococcus Aureus* Bacteraemia in Nasal Carriers versus Non-Carriers," *The Lancet* 364, no. 9435 (August 2004): 703–5, doi:10.1016/S0140-6736(04)16897-9.

74. Mona Johannessen, Johanna E. Sollid, and Anne-Merethe Hanssen, "Host- and Microbe Determinants That May Influence the Success of *S. Aureus* Colonization," *Frontiers in Cellular and Infection Microbiology* 2 (May 4, 2012), doi:10.3389/fcimb.2012.00056.

75. Clara Bueno et al., "T Cell Signalling Induced by Bacterial Superantigens," in *Chemical Immunology and Allergy*, ed. G. Marone (Basel: KARGER, 2007), 161–80, <http://www.karger.com/doi/10.1159/000100894>.

76. Thu A. Chau et al., "Toll-like Receptor 2 Ligands on the Staphylococcal Cell Wall Downregulate Superantigen-Induced T Cell Activation and Prevent Toxic Shock Syndrome," *Nature Medicine* 15, no. 6 (June 2009): 641–48, doi:10.1038/nm.1965.

77. Pascale Cossart, Javier Pizarro-Cerdá, and Marc Lecuit, "Invasion of Mammalian Cells by *Listeria Monocytogenes*: Functional Mimicry to Subvert Cellular Functions," *Trends in Cell Biology* 13, no. 1 (January 2003): 23–31, doi:10.1016/S0962-8924(02)00006-5.

78. Lauren A. Zenewicz and Hao Shen, "Innate and Adaptive Immune Responses to *Listeria Monocytogenes*: A Short Overview," *Microbes and Infection, Forum on Listeria From genomics to pathogenesis*, 9, no. 10 (August 2007): 1208–15, doi:10.1016/j.micinf.2007.05.008.

79. Kyle Rohde et al., "Mycobacterium Tuberculosis and the Environment within the Phagosome," *Immunological Reviews* 219, no. 1 (October 2007): 37–54, doi:10.1111/j.1600-065X.2007.00547.x.

80. Maximiliano G. Gutierrez et al., "Autophagy Is a Defense Mechanism Inhibiting BCG and Mycobacterium Tuberculosis Survival in Infected Macrophages," *Cell* 119, no. 6 (December 2004): 753–66, doi:10.1016/j.cell.2004.11.038.

81. Clifford V Harding, Lakshmi Ramachandra, and Mary Jo Wick, "Interaction of Bacteria with Antigen Presenting Cells: Influences on Antigen Presentation and Antibacterial Immunity," *Current Opinion in Immunology* 15, no. 1 (February 2003): 112–19, doi:10.1016/S0952-7915(02)00008-0.

82. Yu-Jin Jung et al., "Evidence Inconsistent with a Negative Influence of T Helper 2 Cells on Protection Afforded by a Dominant T Helper 1 Response against Mycobacterium

- Tuberculosis Lung Infection in Mice,” *Infection and Immunity* 70, no. 11 (November 1, 2002): 6436–43, doi:10.1128/IAI.70.11.6436-6443.2002.
83. Malanovic N. et al., *Antimicrobial Peptides Targeting Gram-Positive Bacteria*. Pharmaceuticals 2016,
 84. Nargis Khan et al., “Innate Immunity Holding the Flanks until Reinforced by Adaptive Immunity against Mycobacterium Tuberculosis Infection,” *Infectious Diseases*, 2016, 328, doi:10.3389/fmicb.2016.00328.
 85. Piet Gros, Fin J. Milder, and Bert J. C. Janssen, “Complement Driven by Conformational Changes,” *Nature Reviews Immunology* 8, no. 1 (January 2008): 48–58, doi:10.1038/nri2231.
 86. Hwan Keun Kim et al., “Recurrent Infections and Immune Evasion Strategies of Staphylococcus Aureus,” *Current Opinion in Microbiology*, Host—microbe interactions: bacteria, 15, no. 1 (February 2012): 92–99, doi:10.1016/j.mib.2011.10.012.
 87. Alexander Laarman et al., “Complement Inhibition by Gram-Positive Pathogens: Molecular Mechanisms and Therapeutic Implications,” *Journal of Molecular Medicine* 88, no. 2 (January 9, 2010): 115–20, doi:10.1007/s00109-009-0572-y.
 88. W. K. Eddie Ip et al., “Phagocytosis and Phagosome Acidification Are Required for Pathogen Processing and MyD88-Dependent Responses to Staphylococcus Aureus,” *The Journal of Immunology* 184, no. 12 (June 15, 2010): 7071–81, doi:10.4049/jimmunol.1000110.
 89. Johan Bylund et al., “Intracellular Generation of Superoxide by the Phagocyte NADPH Oxidase: How, Where, and What For?,” *Free Radical Biology and Medicine* 49, no. 12 (December 2010): 1834–45, doi:10.1016/j.freeradbiomed.2010.09.016.
 90. Sian L. Stafford et al., “Metal Ions in Macrophage Antimicrobial Pathways: Emerging Roles for Zinc and Copper,” *Bioscience Reports* 33, no. 4 (August 1, 2013): e00049, doi:10.1042/BSR20130014.
 91. S. Searle et al., “Localisation of Nramp1 in Macrophages: Modulation with Activation and Infection,” *Journal of Cell Science* 111, no. 19 (October 1, 1998): 2855–66.

92. "Neutrophil Granules: A Library of Innate Immunity Proteins," accessed October 12, 2016, <http://www.sciencedirect.com.gate2.inist.fr/science/article/pii/S1471490607001561>.
93. Mikkel Faurschou and Niels Borregaard, "Neutrophil Granules and Secretory Vesicles in Inflammation," *Microbes and Infection, Forum in Immunology on neutrophils*, 5, no. 14 (November 2003): 1317–27, doi:10.1016/j.micinf.2003.09.008.
94. McGarry Houghton et al., "Macrophage Elastase Kills Bacteria within Murine Macrophages," *Nature* 460, no. 7255 (July 2009): 637–41, doi:10.1038/nature08181.
95. "Neutrophils Versus Staphylococcus Aureus: A Biological Tug of War - Annual Review of Microbiology, 67(1):629," accessed October 12, 2016, <http://www.annualreviews.org.gate2.inist.fr/doi/10.1146/annurev-micro-092412-155746>.
96. "Staphylococcus Aureus Degrades Neutrophil Extracellular Traps to Promote Immune Cell Death | Science," accessed October 12, 2016, <http://science.sciencemag.org.gate2.inist.fr/content/342/6160/863>.
97. Michail H. Karavolos et al., "Role and Regulation of the Superoxide Dismutases of Staphylococcus Aureus," *Microbiology* 149, no. 10 (2003): 2749–58, doi:10.1099/mic.0.26353-0.
98. Jonathan Kevin Sia, Maria Georgieva, and Jyothi Rengarajan, "Innate Immune Defenses in Human Tuberculosis: An Overview of the Interactions between Mycobacterium Tuberculosis and Innate Immune Cells," *Journal of Immunology Research* 2015 (2015): 1–12, doi:10.1155/2015/747543.
99. Thomas R. Lerner, Sophie Borel, and Maximiliano G. Gutierrez, "The Innate Immune Response in Human Tuberculosis," *Cellular Microbiology* 17, no. 9 (September 2015): 1277–85, doi:10.1111/cmi.12480.
100. "Neutrophils Are the Predominant Infected Phagocytic Cells in the Airways of Patients With Active Pulmonary TB," accessed October 12, 2016, <http://www.sciencedirect.com.gate2.inist.fr/science/article/pii/S0012369210600244>.
101. D. D. Riedel and S. H. Kaufmann, "Chemokine Secretion by Human Polymorphonuclear Granulocytes after Stimulation with Mycobacterium Tuberculosis and Lipoarabinomannan.," *Infection and Immunity* 65, no. 11 (November 1, 1997): 4620–23.

102. Finlay W. McNab et al., "Programmed Death Ligand 1 Is over-Expressed by Neutrophils in the Blood of Patients with Active Tuberculosis," *European Journal of Immunology* 41, no. 7 (July 2011): 1941–47, doi:10.1002/eji.201141421.
103. Ramakrishna Vankayalapati et al., "Role of NK Cell-Activating Receptors and Their Ligands in the Lysis of Mononuclear Phagocytes Infected with an Intracellular Bacterium," *The Journal of Immunology* 175, no. 7 (October 1, 2005): 4611–17, doi:10.4049/jimmunol.175.7.4611.
104. Silvia Cerboni, Matteo Gentili, and Nicolas Manel, "Chapter Eight - Diversity of Pathogen Sensors in Dendritic Cells," in *Advances in Immunology*, ed. Kenneth M. Murphy and Miriam Merad, vol. 120, *Development and Function of Myeloid Subsets* (Academic Press, 2013), 211–37, <http://www.sciencedirect.com/science/article/pii/B9780124170285000089>.
105. Alessandra Cambi, Marjolein Koopman, and Carl G. Figdor, "How C-Type Lectins Detect Pathogens," *Cellular Microbiology* 7, no. 4 (April 1, 2005): 481–88, doi:10.1111/j.1462-5822.2005.00506.x.
106. Marcello Chieppa et al., "Cross-Linking of the Mannose Receptor on Monocyte-Derived Dendritic Cells Activates an Anti-Inflammatory Immunosuppressive Program," *The Journal of Immunology* 171, no. 9 (November 1, 2003): 4552–60, doi:10.4049/jimmunol.171.9.4552.
107. Ludovic Tailleux et al., "DC-SIGN Is the Major Mycobacterium Tuberculosis Receptor on Human Dendritic Cells," *The Journal of Experimental Medicine* 197, no. 1 (January 6, 2003): 121–27, doi:10.1084/jem.20021468.
108. Luis B. Barreiro et al., "Promoter Variation in the DC-SIGN–Encoding Gene CD209 Is Associated with Tuberculosis," *PLOS Med* 3, no. 2 (gen 2006): e20, doi:10.1371/journal.pmed.0030020.
109. Meriem Ben-Ali et al., "Promoter and Neck Region Length Variation of DC-SIGN Is Not Associated with Susceptibility to Tuberculosis in Tunisian Patients," *Human Immunology* 68, no. 11 (November 2007): 908–12, doi:10.1016/j.humimm.2007.09.003.

110. Hye-Mi Lee et al., "Dectin-1 Is Inducible and Plays an Essential Role for Mycobacteria-Induced Innate Immune Responses in Airway Epithelial Cells," *Journal of Clinical Immunology* 29, no. 6 (July 25, 2009): 795, doi:10.1007/s10875-009-9319-3.
111. Eri Ishikawa et al., "Direct Recognition of the Mycobacterial Glycolipid, Trehalose Dimycolate, by C-Type Lectin Mincle," *The Journal of Experimental Medicine* 206, no. 13 (December 21, 2009): 2879–88, doi:10.1084/jem.20091750.
112. Maria Febbraio, David P. Hajjar, and Roy L. Silverstein, "CD36: A Class B Scavenger Receptor Involved in Angiogenesis, Atherosclerosis, Inflammation, and Lipid Metabolism," *Journal of Clinical Investigation* 108, no. 6 (September 15, 2001): 785–91, doi:10.1172/JCI14006.
113. Georg Kraal et al., "The Macrophage Receptor MARCO," *Microbes and Infection* 2, no. 3 (March 2000): 313–16, doi:10.1016/S1286-4579(00)00296-3.
114. Johnathan Canton, Dante Neculai, and Sergio Grinstein, "Scavenger Receptors in Homeostasis and Immunity," *Nature Reviews Immunology* 13, no. 9 (September 2013): 621–34, doi:10.1038/nri3515.
115. Vanesa G. Martinez et al., "The Macrophage Soluble Receptor AIM/Api6/CD5L Displays a Broad Pathogen Recognition Spectrum and Is Involved in Early Response to Microbial Aggression," *Cellular & Molecular Immunology* 11, no. 4 (July 2014): 343–54, doi:10.1038/cmi.2014.12.
116. Jérôme Pugin et al., "CD14 Is a Pattern Recognition Receptor," *Immunity* 1, no. 6 (September 1994): 509–16, doi:10.1016/1074-7613(94)90093-0.
117. Thirumala-Devi Kanneganti, Mohamed Lamkanfi, and Gabriel Núñez, "Intracellular NOD-like Receptors in Host Defense and Disease," *Immunity* 27, no. 4 (October 2007): 549–59, doi:10.1016/j.immuni.2007.10.002.
118. Fabio Martinon, Kimberly Burns, and Jürg Tschopp, "The Inflammasome: A Molecular Platform Triggering Activation of Inflammatory Caspases and Processing of proIL- β ," *Molecular Cell* 10, no. 2 (August 2002): 417–26, doi:10.1016/S1097-2765(02)00599-3.

119. Shizuo Akira, Satoshi Uematsu, and Osamu Takeuchi, "Pathogen Recognition and Innate Immunity," *Cell* 124, no. 4 (February 24, 2006): 783–801, doi:10.1016/j.cell.2006.02.015.
120. Fumitaka Hayashi et al., "The Innate Immune Response to Bacterial Flagellin Is Mediated by Toll-like Receptor 5," *Nature* 410, no. 6832 (April 26, 2001): 1099–1103, doi:10.1038/35074106.
121. "Crystal Structure of the TLR4-MD-2 Complex with Bound Endotoxin Antagonist Eritoran," accessed August 5, 2016, <http://www.sciencedirect.com.gate2.inist.fr/science/article/pii/S0092867407010215>.
122. "Recognition of Double-Stranded RNA and Activation of NF-[kappa]B by Toll-like Receptor 3 : Article : Nature," accessed August 5, 2016, <http://www.nature.com.gate2.inist.fr/nature/journal/v413/n6857/full/413732a0.html>.
123. Sandra S. Diebold et al., "Innate Antiviral Responses by Means of TLR7-Mediated Recognition of Single-Stranded RNA," *Science* 303, no. 5663 (March 5, 2004): 1529–31, doi:10.1126/science.1093616.
124. Hiroaki Hemmi et al., "Small Anti-Viral Compounds Activate Immune Cells via the TLR7 MyD88-dependent Signaling Pathway," *Nature Immunology* 3, no. 2 (February 2002): 196–200, doi:10.1038/ni758.
125. Hiroaki Hemmi et al., "A Toll-like Receptor Recognizes Bacterial DNA," *Nature* 408, no. 6813 (December 2000): 740–45, doi:10.1038/35047123.
126. Uzma Hasan et al., "Human TLR10 Is a Functional Receptor, Expressed by B Cells and Plasmacytoid Dendritic Cells, Which Activates Gene Transcription through MyD88," *The Journal of Immunology* 174, no. 5 (March 1, 2005): 2942–50, doi:10.4049/jimmunol.174.5.2942.
127. Gregory M. Barton, Jonathan C. Kagan, and Ruslan Medzhitov, "Intracellular Localization of Toll-like Receptor 9 Prevents Recognition of Self DNA but Facilitates Access to Viral DNA," *Nature Immunology* 7, no. 1 (January 2006): 49–56, doi:10.1038/ni1280.

128. You-Me Kim et al., "UNC93B1 Delivers Nucleotide-Sensing Toll-like Receptors to Endolysosomes," *Nature* 452, no. 7184 (March 13, 2008): 234–38, doi:10.1038/nature06726.
129. Su-Chang Lin, Yu-Chih Lo, and Hao Wu, "Helical Assembly in the MyD88–IRAK4–IRAK2 Complex in TLR/IL-1R Signalling," *Nature* 465, no. 7300 (June 2010): 885–90, doi:10.1038/nature09121.
130. Maria A. Ermolaeva et al., "Function of TRADD in Tumor Necrosis Factor Receptor 1 Signaling and in TRIF-Dependent Inflammatory Responses," *Nature Immunology* 9, no. 9 (September 2008): 1037–46, doi:10.1038/ni.1638.
131. Mi Sun Jin et al., "Crystal Structure of the TLR1-TLR2 Heterodimer Induced by Binding of a Tri-Acylated Lipopeptide," *Cell* 130, no. 6 (September 2007): 1071–82, doi:10.1016/j.cell.2007.09.008.
132. Grace Soong et al., "TLR2 Is Mobilized into an Apical Lipid Raft Receptor Complex to Signal Infection in Airway Epithelial Cells," *Journal of Clinical Investigation* 113, no. 10 (May 15, 2004): 1482–89, doi:10.1172/JCI20773.
133. Jeroen van Bergenhenegouwen et al., "TLR2 & Co: A Critical Analysis of the Complex Interactions between TLR2 and Coreceptors," *Journal of Leukocyte Biology* 94, no. 5 (November 1, 2013): 885–902, doi:10.1189/jlb.0113003.
134. Takashi Nakata et al., "CD14 Directly Binds to Triacylated Lipopeptides and Facilitates Recognition of the Lipopeptides by the Receptor Complex of Toll-like Receptors 2 and 1 without Binding to the Complex," *Cellular Microbiology* 8, no. 12 (December 2006): 1899–1909, doi:10.1111/j.1462-5822.2006.00756.x.
135. Gisa Gerold et al., "A Toll-like Receptor 2–integrin β 3 Complex Senses Bacterial Lipopeptides via Vitronectin," *Nature Immunology* 9, no. 7 (July 2008): 761–68, doi:10.1038/ni.1618.
136. Hartmut Stoll et al., "Staphylococcus Aureus Deficient in Lipidation of Prelipoproteins Is Attenuated in Growth and Immune Activation," *Infection and Immunity* 73, no. 4 (April 1, 2005): 2411–23, doi:10.1128/IAI.73.4.2411-2423.2005.

137. Jason W. Johnston et al., "Lipoprotein PsaA in Virulence of *Streptococcus Pneumoniae*: Surface Accessibility and Role in Protection from Superoxide," *Infection and Immunity* 72, no. 10 (October 1, 2004): 5858–67, doi:10.1128/IAI.72.10.5858-5867.2004.
138. Shu Okugawa et al., "Lipoprotein Biosynthesis by Prolipoprotein Diacylglycerol Transferase Is Required for Efficient Spore Germination and Full Virulence of *Bacillus Anthracis*," *Molecular Microbiology* 83, no. 1 (January 2012): 96–109, doi:10.1111/j.1365-2958.2011.07915.x.
139. Thomas M. Charlton et al., "Quantitative Lipoproteomics in *Clostridium Difficile* Reveals a Role for Lipoproteins in Sporulation," *Chemistry & Biology* 22, no. 11 (November 19, 2015): 1562–73, doi:10.1016/j.chembiol.2015.10.006.
140. M. Schmollinger et al., "ParSeq: Searching Motifs with Structural and Biochemical Properties," *Bioinformatics* 20, no. 9 (June 12, 2004): 1459–61, doi:10.1093/bioinformatics/bth083.
141. M. Verdrengh and A. Tarkowski, "Role of Neutrophils in Experimental Septicemia and Septic Arthritis Induced by *Staphylococcus Aureus*," *Infection and Immunity* 65, no. 7 (July 1, 1997): 2517–21.
142. Bénédicte Fournier, "The Function of TLR2 during Staphylococcal Diseases," *Frontiers in Cellular and Infection Microbiology* 2 (January 4, 2013), doi:10.3389/fcimb.2012.00167.
143. Dong-Min Shin et al., "Mycobacterium Tuberculosis Lipoprotein-Induced Association of TLR2 with Protein Kinase C ζ in Lipid Rafts Contributes to Reactive Oxygen Species-Dependent Inflammatory Signalling in Macrophages," *Cellular Microbiology* 10, no. 9 (September 2008): 1893–1905, doi:10.1111/j.1462-5822.2008.01179.x.
144. Dong-Min Shin et al., "Mycobacterial Lipoprotein Activates Autophagy via TLR2/1/CD14 and a Functional Vitamin D Receptor Signalling," *Cellular Microbiology* 12, no. 11 (November 1, 2010): 1648–65, doi:10.1111/j.1462-5822.2010.01497.x.
145. Philip T. Liu et al., "Convergence of IL-1 β and VDR Activation Pathways in Human TLR2/1-Induced Antimicrobial Responses," *PLOS ONE* 4, no. 6 (ju 2009): e5810, doi:10.1371/journal.pone.0005810.

146. Deepti Gupta et al., "Suppression of TLR2-Induced IL-12, Reactive Oxygen Species, and Inducible Nitric Oxide Synthase Expression by Mycobacterium Tuberculosis Antigens Expressed inside Macrophages during the Course of Infection," *The Journal of Immunology* 184, no. 10 (May 15, 2010): 5444–55, doi:10.4049/jimmunol.0903283.
147. Shiny Nair et al., "The PPE18 of Mycobacterium Tuberculosis Interacts with TLR2 and Activates IL-10 Induction in Macrophage," *The Journal of Immunology* 183, no. 10 (November 15, 2009): 6269–81, doi:10.4049/jimmunol.0901367.
148. Nooruddin Khan et al., "Mycobacterium Tuberculosis Heat Shock Protein 60 Modulates Immune Response to PPD by Manipulating the Surface Expression of TLR2 on Macrophages," *Cellular Microbiology* 10, no. 8 (August 2008): 1711–22, doi:10.1111/j.1462-5822.2008.01161.x.
149. Rafael Prados-Rosales et al., "Mycobacteria Release Active Membrane Vesicles That Modulate Immune Responses in a TLR2-Dependent Manner in Mice," *Journal of Clinical Investigation* 121, no. 4 (April 1, 2011): 1471–83, doi:10.1172/JCI44261.
150. Caroline Hémond et al., "Human Blood mDC Subsets Exhibit Distinct TLR Repertoire and Responsiveness," *Journal of Leukocyte Biology* 93, no. 4 (April 1, 2013): 599–609, doi:10.1189/jlb.0912452.
151. Yi Yang et al., "Heat Shock Protein gp96 Is a Master Chaperone for Toll-like Receptors and Is Important in the Innate Function of Macrophages," *Immunity* 26, no. 2 (February 23, 2007): 215–26, doi:10.1016/j.immuni.2006.12.005.
152. Jennifer M. Lund et al., "Recognition of Single-Stranded RNA Viruses by Toll-like Receptor 7," *Proceedings of the National Academy of Sciences of the United States of America* 101, no. 15 (April 13, 2004): 5598–5603, doi:10.1073/pnas.0400937101.
153. Tobias Haas et al., "The DNA Sugar Backbone 2' Deoxyribose Determines Toll-like Receptor 9 Activation," *Immunity* 28, no. 3 (March 14, 2008): 315–23, doi:10.1016/j.immuni.2008.01.013.
154. Kun Yang et al., "Human TLR-7-, -8-, and -9-Mediated Induction of IFN- α/β and - λ Is IRAK-4 Dependent and Redundant for Protective Immunity to Viruses," *Immunity* 23, no. 5 (November 2005): 465–78, doi:10.1016/j.immuni.2005.09.016.

155. Taro Kawai et al., "Interferon- α Induction through Toll-like Receptors Involves a Direct Interaction of IRF7 with MyD88 and TRAF6," *Nature Immunology* 5, no. 10 (October 2004): 1061–68, doi:10.1038/ni1118.
156. Cristiana Guiducci et al., "PI3K Is Critical for the Nuclear Translocation of IRF-7 and Type I IFN Production by Human Plasmacytoid Predendritic Cells in Response to TLR Activation," *The Journal of Experimental Medicine* 205, no. 2 (February 18, 2008): 315–22, doi:10.1084/jem.20070763.
157. Prafullakumar Tailor et al., "The Feedback Phase of Type I Interferon Induction in Dendritic Cells Requires Interferon Regulatory Factor 8," *Immunity* 27, no. 2 (August 2007): 228–39, doi:10.1016/j.immuni.2007.06.009.
158. Kenya Honda et al., "Spatiotemporal Regulation of MyD88–IRF-7 Signalling for Robust Type-I Interferon Induction," *Nature* 434, no. 7036 (April 21, 2005): 1035–40, doi:10.1038/nature03547.
159. Wei Cao et al., "Plasmacytoid Dendritic Cell-specific Receptor ILT7–Fc ϵ R1 γ Inhibits Toll-like Receptor-induced Interferon Production," *The Journal of Experimental Medicine* 203, no. 6 (June 12, 2006): 1399–1405, doi:10.1084/jem.20052454.
160. Andrzej Dzionek et al., "BDCA-2, a Novel Plasmacytoid Dendritic Cell-specific Type II C-Type Lectin, Mediates Antigen Capture and Is a Potent Inhibitor of Interferon A/ β Induction," *The Journal of Experimental Medicine* 194, no. 12 (December 17, 2001): 1823–34, doi:10.1084/jem.194.12.1823.
161. Amanda L. Blasius et al., "Siglec-H Is an IPC-Specific Receptor That Modulates Type I IFN Secretion through DAP12," *Blood* 107, no. 6 (March 15, 2006): 2474–76, doi:10.1182/blood-2005-09-3746.
162. Anja Fuchs et al., "Paradoxical Inhibition of Human Natural Interferon-Producing Cells by the Activating Receptor NKp44," *Blood* 106, no. 6 (September 15, 2005): 2076–82, doi:10.1182/blood-2004-12-4802.
163. Sidney Pestka, Christopher D. Krause, and Mark R. Walter, "Interferons, Interferon-like Cytokines, and Their Receptors," *Immunological Reviews* 202, no. 1 (December 2004): 8–32, doi:10.1111/j.0105-2896.2004.00204.x.

164. Jamie R. Schoenborn and Christopher B. Wilson, "Regulation of Interferon- γ During Innate and Adaptive Immune Responses," ed. BT - Advances in Immunology, vol. 96 (Academic Press, 2007), 41–101, <http://www.sciencedirect.com/science/article/pii/S0065277607960022>.
165. Thomas R. O'Brien, Ludmila Prokunina-Olsson, and Raymond P. Donnelly, "IFN- λ 4: The Paradoxical New Member of the Interferon Lambda Family," *Journal of Interferon & Cytokine Research* 34, no. 11 (April 30, 2014): 829–38, doi:10.1089/jir.2013.0136.
166. Kenya Honda, Akinori Takaoka, and Tadatsugu Taniguchi, "Type I Interferon Gene Induction by the Interferon Regulatory Factor Family of Transcription Factors," *Immunity* 25, no. 3 (September 2006): 349–60, doi:10.1016/j.immuni.2006.08.009.
167. Lionel B. Ivashkiv and Laura T. Donlin, "Regulation of Type I Interferon Responses," *Nature Reviews Immunology* 14, no. 1 (January 2014): 36–49, doi:10.1038/nri3581.
168. Nan Yan and Zhijian J. Chen, "Intrinsic Antiviral Immunity," *Nature Immunology* 13, no. 3 (March 2012): 214–22, doi:10.1038/ni.2229.
169. Gijs A Versteeg and Adolfo García-Sastre, "Viral Tricks to Grid-Lock the Type I Interferon System," *Current Opinion in Microbiology, Host–Microbe Interactions: Fungi/Parasites/Viruses*, 13, no. 4 (August 2010): 508–16, doi:10.1016/j.mib.2010.05.009.
170. Jean-Laurent Casanova, Steven M. Holland, and Luigi D. Notarangelo, "Inborn Errors of Human JAKs and STATs," *Immunity* 36, no. 4 (April 20, 2012): 515–28, doi:10.1016/j.immuni.2012.03.016.
171. O. Haller et al., "Virus-Specific Interferon Action. Protection of Newborn Mx Carriers against Lethal Infection with Influenza Virus," *The Journal of Experimental Medicine* 154, no. 1 (July 1, 1981): 199–203, doi:10.1084/jem.154.1.199.
172. U. Muller et al., "Functional Role of Type I and Type II Interferons in Antiviral Defense," *Science* 264, no. 5167 (June 24, 1994): 1918–21, doi:10.1126/science.8009221.
173. Netanya G. Sandler et al., "Type I Interferon Responses in Rhesus Macaques Prevent SIV Infection and Slow Disease Progression," *Nature* 511, no. 7511 (July 2014): 601–5, doi:10.1038/nature13554.

174. Marc Dauer et al., "Interferon- α Disables Dendritic Cell Precursors: Dendritic Cells Derived from Interferon- α -Treated Monocytes Are Defective in Maturation and T-Cell Stimulation," *Immunology* 110, no. 1 (September 2003): 38–47, doi:10.1046/j.1365-2567.2003.01702.x.
175. Bumsuk Hahm et al., "Viruses Evade the Immune System through Type I Interferon-Mediated STAT2-Dependent, but STAT1-Independent, Signaling," *Immunity* 22, no. 2 (February 2005): 247–57, doi:10.1016/j.immuni.2005.01.005.
176. Tomoki Ito et al., "Differential Regulation of Human Blood Dendritic Cell Subsets by IFNs," *The Journal of Immunology* 166, no. 5 (March 1, 2001): 2961–69, doi:10.4049/jimmunol.166.5.2961.
177. Maria Montoya et al., "Type I Interferons Produced by Dendritic Cells Promote Their Phenotypic and Functional Activation," *Blood* 99, no. 9 (May 1, 2002): 3263–71, doi:10.1182/blood.V99.9.3263.
178. Agnes Le Bon et al., "Cross-Priming of CD8+ T Cells Stimulated by Virus-Induced Type I Interferon," *Nature Immunology* 4, no. 10 (October 2003): 1009–15, doi:10.1038/ni978.
179. Francesca Spadaro et al., "IFN- α Enhances Cross-Presentation in Human Dendritic Cells by Modulating Antigen Survival, Endocytic Routing, and Processing," *Blood* 119, no. 6 (February 9, 2012): 1407–17, doi:10.1182/blood-2011-06-363564.
180. Grégory Gautier et al., "A Type I Interferon Autocrine–paracrine Loop Is Involved in Toll-like Receptor-Induced Interleukin-12p70 Secretion by Dendritic Cells," *The Journal of Experimental Medicine* 201, no. 9 (May 2, 2005): 1435–46, doi:10.1084/jem.20041964.
181. Sophia Davidson et al., "Pathogenic Potential of Interferon A β in Acute Influenza Infection," *Nature Communications* 5 (May 2014): 3864, doi:10.1038/ncomms4864.
182. Andrew W. Hardy et al., "HIV Turns Plasmacytoid Dendritic Cells (pDC) into TRAIL-Expressing Killer pDC and down-Regulates HIV Coreceptors by Toll-like Receptor 7-Induced IFN- α ," *Proceedings of the National Academy of Sciences* 104, no. 44 (October 30, 2007): 17453–58, doi:10.1073/pnas.0707244104.

183. Jean-Philippe Herbeuval et al., "CD4+ T-Cell Death Induced by Infectious and Noninfectious HIV-1: Role of Type 1 Interferon-dependent, TRAIL/DR5-Mediated Apoptosis," *Blood* 106, no. 10 (November 15, 2005): 3524–31, doi:10.1182/blood-2005-03-1243.
184. Lucas J. Thompson et al., "Innate Inflammatory Signals Induced by Various Pathogens Differentially Dictate the IFN-I Dependence of CD8 T Cells for Clonal Expansion and Memory Formation," *The Journal of Immunology* 177, no. 3 (August 1, 2006): 1746–54, doi:10.4049/jimmunol.177.3.1746.
185. Ramon Gimeno et al., "Stat1 and Stat2 but Not Stat3 Arbitrate Contradictory Growth Signals Elicited by Alpha/Beta Interferon in T Lymphocytes," *Molecular and Cellular Biology* 25, no. 13 (July 1, 2005): 5456–65, doi:10.1128/MCB.25.13.5456-5465.2005.
186. Ilwoong Hwang et al., "Activation Mechanisms of Natural Killer Cells during Influenza Virus Infection," *PLOS ONE* 7, no. 12 (dic 2012): e51858, doi:10.1371/journal.pone.0051858.
187. Josh Crouse et al., "Type I Interferons Protect T Cells against NK Cell Attack Mediated by the Activating Receptor NCR1," *Immunity* 40, no. 6 (June 2014): 961–73, doi:10.1016/j.immuni.2014.05.003.
188. Katja Fink et al., "Early Type I Interferon-Mediated Signals on B Cells Specifically Enhance Antiviral Humoral Responses," *European Journal of Immunology* 36, no. 8 (August 2006): 2094–2105, doi:10.1002/eji.200635993.
189. Tatsuya Ishihara et al., "Inhibition of *Chlamydia trachomatis* Growth by Human Interferon- α : Mechanisms and Synergistic Effect with Interferon- γ and Tumor Necrosis Factor- α ," *Biomedical Research* 26, no. 4 (2005): 179–85, doi:10.2220/biomedres.26.179.
190. Giuseppe Mancuso et al., "Type I IFN Signaling Is Crucial for Host Resistance against Different Species of Pathogenic Bacteria," *The Journal of Immunology* 178, no. 5 (March 1, 2007): 3126–33, doi:10.4049/jimmunol.178.5.3126.

191. Giovanna Schiavoni et al., "Type I IFN Protects Permissive Macrophages from *Legionella Pneumophila* Infection through an IFN- γ -Independent Pathway," *The Journal of Immunology* 173, no. 2 (July 15, 2004): 1266–75, doi:10.4049/jimmunol.173.2.1266.
192. Douglas A. Weigent et al., "Role of Interferon in Streptococcal Infection in the Mouse," *Microbial Pathogenesis* 1, no. 4 (August 1986): 399–407, doi:10.1016/0882-4010(86)90071-9.
193. Victoria Auerbuch et al., "Mice Lacking the Type I Interferon Receptor Are Resistant to *Listeria Monocytogenes*," *The Journal of Experimental Medicine* 200, no. 4 (August 16, 2004): 527–33, doi:10.1084/jem.20040976.
194. Javier A. Carrero, Boris Calderon, and Emil R. Unanue, "Type I Interferon Sensitizes Lymphocytes to Apoptosis and Reduces Resistance to *Listeria* Infection," *The Journal of Experimental Medicine* 200, no. 4 (August 16, 2004): 535–40, doi:10.1084/jem.20040769.
195. Ryan M. O'Connell et al., "Type I Interferon Production Enhances Susceptibility to *Listeria Monocytogenes* Infection," *The Journal of Experimental Medicine* 200, no. 4 (August 16, 2004): 437–45, doi:10.1084/jem.20040712.
196. Javier A. Carrero, Boris Calderon, and Emil R. Unanue, "Lymphocytes Are Detrimental during the Early Innate Immune Response against *Listeria Monocytogenes*," *The Journal of Experimental Medicine* 203, no. 4 (April 17, 2006): 933–40, doi:10.1084/jem.20060045.
197. Silvia Stockinger et al., "Production of Type I IFN Sensitizes Macrophages to Cell Death Induced by *Listeria Monocytogenes*," *The Journal of Immunology* 169, no. 11 (December 1, 2002): 6522–29, doi:10.4049/jimmunol.169.11.6522.
198. Heather Zwaferink et al., "IFN- β Increases Listeriolysin O-Induced Membrane Permeabilization and Death of Macrophages," *The Journal of Immunology* 180, no. 6 (March 15, 2008): 4116–23, doi:10.4049/jimmunol.180.6.4116.
199. Heather Zwaferink et al., "Stimulation of Inducible Nitric Oxide Synthase Expression by Beta Interferon Increases Necrotic Death of Macrophages upon *Listeria Monocytogenes* Infection," *Infection and Immunity* 76, no. 4 (April 1, 2008): 1649–56, doi:10.1128/IAI.01251-07.

200. Manira Rayamajhi et al., "Induction of IFN- $\alpha\beta$ Enables *Listeria Monocytogenes* to Suppress Macrophage Activation by IFN- γ ," *The Journal of Experimental Medicine* 207, no. 2 (February 15, 2010): 327–37, doi:10.1084/jem.20091746.
201. Matthew P. R. Berry et al., "An Interferon-Inducible Neutrophil-Driven Blood Transcriptional Signature in Human Tuberculosis," *Nature* 466, no. 7309 (August 2010): 973–77, doi:10.1038/nature09247.
202. Lis R.V. Antonelli et al., "Intranasal Poly-IC Treatment Exacerbates Tuberculosis in Mice through the Pulmonary Recruitment of a Pathogen-Permissive Monocyte/macrophage Population," *Journal of Clinical Investigation* 120, no. 5 (May 3, 2010): 1674–82, doi:10.1172/JCI40817.
203. Claudia Manca et al., "Hypervirulent *M. Tuberculosis* W/Beijing Strains Upregulate Type I IFNs and Increase Expression of Negative Regulators of the Jak-Stat Pathway," *Journal of Interferon & Cytokine Research* 25, no. 11 (November 1, 2005): 694–701, doi:10.1089/jir.2005.25.694.
204. Claudia Manca et al., "Virulence of a *Mycobacterium Tuberculosis* Clinical Isolate in Mice Is Determined by Failure to Induce Th1 Type Immunity and Is Associated with Induction of IFN- α/β ," *Proceedings of the National Academy of Sciences* 98, no. 10 (May 8, 2001): 5752–57, doi:10.1073/pnas.091096998.
205. Jacqueline M. Cliff et al., "Distinct Phases of Blood Gene Expression Pattern Through Tuberculosis Treatment Reflect Modulation of the Humoral Immune Response," *Journal of Infectious Diseases* 207, no. 1 (January 1, 2013): 18–29, doi:10.1093/infdis/jis499.
206. Tom H. M. Ottenhoff et al., "Genome-Wide Expression Profiling Identifies Type 1 Interferon Response Pathways in Active Tuberculosis," *PLOS ONE* 7, no. 9 (set 2012): e45839, doi:10.1371/journal.pone.0045839.
207. Katrin D. Mayer-Barber et al., "Innate and Adaptive Interferons Suppress IL-1 α and IL-1 β Production by Distinct Pulmonary Myeloid Subsets during *Mycobacterium Tuberculosis* Infection," *Immunity* 35, no. 6 (December 2011): 1023–34, doi:10.1016/j.immuni.2011.12.002.
208. Finlay W. McNab et al., "Type I IFN Induces IL-10 Production in an IL-27–Independent Manner and Blocks Responsiveness to IFN- γ for Production of IL-12 and

- Bacterial Killing in *Mycobacterium tuberculosis*–Infected Macrophages,” *The Journal of Immunology* 193, no. 7 (October 1, 2014): 3600–3612, doi:10.4049/jimmunol.1401088.
209. Greta Guarda et al., “Type I Interferon Inhibits Interleukin-1 Production and Inflammasome Activation,” *Immunity* 34, no. 2 (February 25, 2011): 213–23, doi:10.1016/j.immuni.2011.02.006.
 210. Roelof A. de Paus et al., “Inhibition of the Type I Immune Responses of Human Monocytes by IFN- α and IFN- β ,” *Cytokine* 61, no. 2 (February 2013): 645–55, doi:10.1016/j.cyto.2012.12.005.
 211. Finlay W. McNab et al., “TPL-2–ERK1/2 Signaling Promotes Host Resistance against Intracellular Bacterial Infection by Negative Regulation of Type I IFN Production,” *The Journal of Immunology* 191, no. 4 (August 15, 2013): 1732–43, doi:10.4049/jimmunol.1300146.
 212. Katrin D. Mayer-Barber et al., “Host-Directed Therapy of Tuberculosis Based on Interleukin-1 and Type I Interferon Crosstalk,” *Nature* 511, no. 7507 (July 2014): 99–103, doi:10.1038/nature13489.
 213. Rosane M. B. Teles et al., “Type I Interferon Suppresses Type II Interferon–Triggered Human Anti-*Mycobacterial* Responses,” *Science* 339, no. 6126 (March 22, 2013): 1448–53, doi:10.1126/science.1233665.
 214. Yasmine Belkaid and Timothy W. Hand, “Role of the Microbiota in Immunity and Inflammation,” *Cell* 157, no. 1 (March 27, 2014): 121–41, doi:10.1016/j.cell.2014.03.011.
 215. Daniel J. Gough et al., “Constitutive Type I Interferon Modulates Homeostatic Balance through Tonic Signaling,” *Immunity* 36, no. 2 (February 24, 2012): 166–74, doi:10.1016/j.immuni.2012.01.011.
 216. Stephanie C. Ganal et al., “Priming of Natural Killer Cells by Nonmucosal Mononuclear Phagocytes Requires Instructive Signals from Commensal Microbiota,” *Immunity* 37, no. 1 (July 2012): 171–86, doi:10.1016/j.immuni.2012.05.020.

217. Markus Tschurtschenthaler et al., "Type I Interferon Signalling in the Intestinal Epithelium Affects Paneth Cells, Microbial Ecology and Epithelial Regeneration," *Gut* 63, no. 12 (December 1, 2014): 1921–31, doi:10.1136/gutjnl-2013-305863.
218. David M. Morens, Jeffery K. Taubenberger, and Anthony S. Fauci, "Predominant Role of Bacterial Pneumonia as a Cause of Death in Pandemic Influenza: Implications for Pandemic Influenza Preparedness," *Journal of Infectious Diseases* 198, no. 7 (October 1, 2008): 962–70, doi:10.1086/591708.
219. Shigeki Nakamura, Kimberly M. Davis, and Jeffrey N. Weiser, "Synergistic Stimulation of Type I Interferons during Influenza Virus Coinfection Promotes *Streptococcus Pneumoniae* Colonization in Mice," *Journal of Clinical Investigation* 121, no. 9 (September 1, 2011): 3657–65, doi:10.1172/JCI57762.
220. Alexander A. Navarini et al., "Increased Susceptibility to Bacterial Superinfection as a Consequence of Innate Antiviral Responses," *Proceedings of the National Academy of Sciences* 103, no. 42 (October 17, 2006): 15535–39, doi:10.1073/pnas.0607325103.
221. Lijun Xin et al., "Type I IFN Receptor Regulates Neutrophil Functions and Innate Immunity to *Leishmania* Parasites," *The Journal of Immunology* 184, no. 12 (June 15, 2010): 7047–56, doi:10.4049/jimmunol.0903273.
222. Ashraful Haque et al., "Type I Interferons Suppress CD4+ T-Cell-Dependent Parasite Control during Blood-Stage *Plasmodium* Infection," *European Journal of Immunology* 41, no. 9 (September 2011): 2688–98, doi:10.1002/eji.201141539.
223. Ana Margarida Vigário et al., "Recombinant Human IFN- α Inhibits Cerebral Malaria and Reduces Parasite Burden in Mice," *The Journal of Immunology* 178, no. 10 (May 15, 2007): 6416–25, doi:10.4049/jimmunol.178.10.6416.
224. Carmelo Biondo et al., "IFN- α/β Signaling Is Required for Polarization of Cytokine Responses toward a Protective Type 1 Pattern during Experimental Cryptococcosis," *The Journal of Immunology* 181, no. 1 (July 1, 2008): 566–73, doi:10.4049/jimmunol.181.1.566.
225. Frank L. van de Veerdonk et al., "STAT1 Mutations in Autosomal Dominant Chronic Mucocutaneous Candidiasis," *New England Journal of Medicine* 365, no. 1 (July 2011): 54–61, doi:10.1056/NEJMoa1100102.

226. Paula Michea et al., "Epithelial Control of the Human pDC Response to Extracellular Bacteria," *European Journal of Immunology* 43, no. 5 (May 2013): 1264–73, doi:10.1002/eji.201242990.
227. Marijo Parcina et al., "Pathogen-Triggered Activation of Plasmacytoid Dendritic Cells Induces IL-10–Producing B Cells in Response to *Staphylococcus Aureus*," *The Journal of Immunology* 190, no. 4 (February 15, 2013): 1591–1602, doi:10.4049/jimmunol.1201222.
228. Amy L. Anderson et al., "Pathogenesis of B-Cell Superantigen-Induced Immune Complex-Mediated Inflammation," *Infection and Immunity* 74, no. 2 (February 1, 2006): 1196–1203, doi:10.1128/IAI.74.2.1196-1203.2006.
229. Emily Bond et al., "Plasmacytoid Dendritic Cells Infiltrate the Skin in Positive Tuberculin Skin Test Indurations," *Journal of Investigative Dermatology* 132, no. 1 (January 2012): 114–23, doi:10.1038/jid.2011.246.
230. Laura Lozza et al., "Communication between Human Dendritic Cell Subsets in Tuberculosis: Requirements for Naive CD4+ T Cell Stimulation," *Microbial Immunology* 5 (2014): 324, doi:10.3389/fimmu.2014.00324.
231. M. I Rabinovich and H. D. I Abarbanel, "The Role of Chaos in Neural Systems," *Neuroscience* 87, no. 1 (June 1998): 5–14, doi:10.1016/S0306-4522(98)00091-8.
232. Amit Joshi and Deliang Cao, "TGF-Beta Signaling, Tumor Microenvironment and Tumor Progression: The Butterfly Effect," *Frontiers in Bioscience (Landmark Edition)* 15 (2010): 180–94.
233. Julien Guizetti and Artur Scherf, "Silence, Activate, Poise and Switch! Mechanisms of Antigenic Variation in *Plasmodium Falciparum*," *Cellular Microbiology* 15, no. 5 (May 2013): 718–26, doi:10.1111/cmi.12115.
234. Jonathan P. Richardson and David L. Moyes, "Adaptive Immune Responses to *Candida Albicans* Infection," *Virulence* 6, no. 4 (May 2015): 327–37, doi:10.1080/21505594.2015.1004977.

235. G. T. Seah, G. M. Scott, and G. a. W. Rook, "Type 2 Cytokine Gene Activation and Its Relationship to Extent of Disease in Patients with Tuberculosis," *Journal of Infectious Diseases* 181, no. 1 (January 1, 2000): 385–89, doi:10.1086/315200.
236. Howard and Zwillig, "Reactivation of Tuberculosis Is Associated with a Shift from Type 1 to Type 2 Cytokines," *Clinical & Experimental Immunology* 115, no. 3 (March 1, 1999): 428–34, doi:10.1046/j.1365-2249.1999.00791.x.
237. James Harris et al., "T Helper 2 Cytokines Inhibit Autophagic Control of Intracellular Mycobacterium Tuberculosis," *Immunity* 27, no. 3 (September 21, 2007): 505–17, doi:10.1016/j.immuni.2007.07.022.
238. Bushra Jamil et al., "Interferon- γ /IL10 Ratio Defines the Disease Severity in Pulmonary and Extra Pulmonary Tuberculosis," *Tuberculosis* 87, no. 4 (July 1, 2007): 279–87, doi:10.1016/j.tube.2007.03.004.
239. Jonathan M. Coquet, Lisa Rausch, and Jannie Borst, "The Importance of Co-Stimulation in the Orchestration of T Helper Cell Differentiation," *Immunology and Cell Biology* 93, no. 9 (October 2015): 780–88, doi:10.1038/icb.2015.45.
240. Hyung-Seok Choi et al., "Analysis of Nitric Oxide Synthase and Nitrotyrosine Expression in Human Pulmonary Tuberculosis," *American Journal of Respiratory and Critical Care Medicine* 166, no. 2 (July 2002): 178–86, doi:10.1164/rccm.2201023.
241. Zhi-Jian Ye et al., "Differentiation and Recruitment of Th9 Cells Stimulated by Pleural Mesothelial Cells in Human Mycobacterium Tuberculosis Infection," *PLOS ONE* 7, no. 2 (February 20, 2012): e31710, doi:10.1371/journal.pone.0031710.
242. Dorothee Schmid and Christian Münz, "Innate and Adaptive Immunity through Autophagy," *Immunity* 27, no. 1 (July 27, 2007): 11–21, doi:10.1016/j.immuni.2007.07.004.
243. R Hernández-Pando et al., "Correlation between the Kinetics of Th1, Th2 Cells and Pathology in a Murine Model of Experimental Pulmonary Tuberculosis," *Immunology* 89, no. 1 (September 1996): 26–33.

244. Flávio V. Loures et al., "Recognition of *Aspergillus Fumigatus* Hyphae by Human Plasmacytoid Dendritic Cells Is Mediated by Dectin-2 and Results in Formation of Extracellular Traps," *PLOS Pathog* 11, no. 2 (February 6, 2015): e1004643, doi:10.1371/journal.ppat.1004643.
245. Zaida G. Ramirez-Ortiz et al., "A Nonredundant Role for Plasmacytoid Dendritic Cells in Host Defense against the Human Fungal Pathogen *Aspergillus Fumigatus*," *Cell Host & Microbe* 9, no. 5 (May 2011): 415–24, doi:10.1016/j.chom.2011.04.007.
246. Volker Brinkmann et al., "Neutrophil Extracellular Traps Kill Bacteria," *Science* 303, no. 5663 (March 5, 2004): 1532–35, doi:10.1126/science.1092385.
247. Constantin F. Urban et al., "Neutrophil Extracellular Traps Capture and Kill *Candida Albicans* Yeast and Hyphal Forms," *Cellular Microbiology* 8, no. 4 (April 1, 2006): 668–76, doi:10.1111/j.1462-5822.2005.00659.x.
248. Min Wang et al., "Microbial Hijacking of Complement–Toll-like Receptor Crosstalk," *Science Signaling* 3, no. 109 (February 16, 2010): ra11, doi:10.1126/scisignal.2000697.
249. Hideo Negishi et al., "Negative Regulation of Toll-like-Receptor Signaling by IRF-4," *Proceedings of the National Academy of Sciences of the United States of America* 102, no. 44 (November 1, 2005): 15989–94, doi:10.1073/pnas.0508327102.
250. Mark Gilchrist et al., "Systems Biology Approaches Identify ATF3 as a Negative Regulator of Toll-like Receptor 4," *Nature* 441, no. 7090 (May 2006): 173–78, doi:10.1038/nature04768.
251. Hirotaka Kuwata et al., "IkBNS Inhibits Induction of a Subset of Toll-like Receptor-Dependent Genes and Limits Inflammation," *Immunity* 24, no. 1 (January 2006): 41–51, doi:10.1016/j.immuni.2005.11.004.
252. Ashley Mansell et al., "Suppressor of Cytokine Signaling 1 Negatively Regulates Toll-like Receptor Signaling by Mediating Mal Degradation," *Nature Immunology* 7, no. 2 (February 2006): 148–55, doi:10.1038/ni1299.

253. Hisaaki Shinohara et al., "Dok-1 and Dok-2 Are Negative Regulators of Lipopolysaccharide-Induced Signaling," *The Journal of Experimental Medicine* 201, no. 3 (February 7, 2005): 333–39, doi:10.1084/jem.20041817.
254. Yaya Wang et al., "Association of β -Arrestin and TRAF6 Negatively Regulates Toll-like Receptor–interleukin 1 Receptor Signaling," *Nature Immunology* 7, no. 2 (February 2006): 139–47, doi:10.1038/ni1294.
255. Jian Jun Gao et al., "Cutting Edge: Bacterial DNA and LPS Act in Synergy in Inducing Nitric Oxide Production in RAW 264.7 Macrophages," *The Journal of Immunology* 163, no. 8 (October 15, 1999): 4095–99.
256. Shintaro Sato et al., "Synergy and Cross-Tolerance Between Toll-Like Receptor (TLR) 2- and TLR4-Mediated Signaling Pathways," *The Journal of Immunology* 165, no. 12 (December 15, 2000): 7096–7101, doi:10.4049/jimmunol.165.12.7096.
257. Fumiko Nomura et al., "Cutting Edge: Endotoxin Tolerance in Mouse Peritoneal Macrophages Correlates with Down-Regulation of Surface Toll-Like Receptor 4 Expression," *The Journal of Immunology* 164, no. 7 (April 1, 2000): 3476–79, doi:10.4049/jimmunol.164.7.3476.
258. J. S. Cowdery et al., "Bacterial DNA Induces NK Cells to Produce IFN-Gamma in Vivo and Increases the Toxicity of Lipopolysaccharides.," *The Journal of Immunology* 156, no. 12 (June 15, 1996): 4570–75.
259. Kate Schroder, Matthew J. Sweet, and David A. Hume, "Signal Integration between IFN γ and TLR Signalling Pathways in Macrophages," *Immunobiology, European Macrophage and Dendritic Cell Society*, 211, no. 6–8 (September 2006): 511–24, doi:10.1016/j.imbio.2006.05.007.
260. Mark P. Hayes, Sherry L. Freeman, and Raymond P. Donnelly, "IFN- γ Priming of Monocytes Enhances LPS-Induced TNF Production by Augmenting Both Transcription and mRNA Stability," *Cytokine* 7, no. 5 (July 1995): 427–35, doi:10.1006/cyto.1995.0058.
261. Jennifer Major, Julia E. Fletcher, and Thomas A. Hamilton, "IL-4 Pretreatment Selectively Enhances Cytokine and Chemokine Production in Lipopolysaccharide-Stimulated Mouse Peritoneal Macrophages," *The Journal of Immunology* 168, no. 5 (March 1, 2002): 2456–63, doi:10.4049/jimmunol.168.5.2456.

262. Takuji Hanamura, Eiji Asakura, and Toshizumi Tanabe, "Macrophage Colony-Stimulating Factor (M-CSF) Augments Cytokine Induction by Lipopolysaccharide (LPS)-Stimulation and by Bacterial Infections in Mice," *Immunopharmacology* 37, no. 1 (August 1997): 15–23, doi:10.1016/S0162-3109(96)00166-X.
263. Robert I. Scheinman et al., "Role of Transcriptional Activation of I κ B α in Mediation of Immunosuppression by Glucocorticoids," *Science* 270, no. 5234 (October 13, 1995): 283–86, doi:10.1126/science.270.5234.283.
264. Antonio Cappuccio et al., "Combinatorial Code Governing Cellular Responses to Complex Stimuli," *Nature Communications* 6 (April 21, 2015): 6847, doi:10.1038/ncomms7847.
265. Jean-Laurent Casanova and Laurent Abel, "GENETIC DISSECTION OF IMMUNITY TO MYCOBACTERIA: The Human Model," *Annual Review of Immunology* 20, no. 1 (2002): 581–620, doi:10.1146/annurev.immunol.20.081501.125851.
266. Graham S. Cooke and Adrian V. S. Hill, "Genetics of Susceptibility to Human Infectious Disease," *Nature Reviews Genetics* 2, no. 12 (December 2001): 967–77, doi:10.1038/35103577.
267. N. T. T. Thuong et al., "A Polymorphism in Human TLR2 Is Associated with Increased Susceptibility to Tuberculous Meningitis," *Genes and Immunity* 8, no. 5 (June 2007): 422–28, doi:10.1038/sj.gene.6364405.
268. Pedro Pimentel-Nunes et al., "Functional Polymorphisms of Toll-like Receptors 2 and 4 Alter the Risk for Colorectal Carcinoma in Europeans," *Digestive and Liver Disease* 45, no. 1 (January 2013): 63–69, doi:10.1016/j.dld.2012.08.006.
269. Tannette G. Krediet et al., "Toll-like Receptor 2 Polymorphism Is Associated With Preterm Birth," *Pediatric Research* 62, no. 4 (October 2007): 474–76, doi:10.1203/PDR.0b013e31813c9401.
270. Saumya Pandey et al., "Impact of Toll-like Receptors [TLR] 2 (–196 to –174 Del) and TLR 4 (Asp299Gly, Thr399Ile) in Cervical Cancer Susceptibility in North Indian Women," *Gynecologic Oncology* 114, no. 3 (September 2009): 501–5, doi:10.1016/j.ygyno.2009.05.032.

271. Tomomitsu Tahara et al., "Toll-like Receptor 2 –196 to 174del Polymorphism Influences the Susceptibility of Japanese People to Gastric Cancer," *Cancer Science* 98, no. 11 (November 1, 2007): 1790–94, doi:10.1111/j.1349-7006.2007.00590.x.
272. Yuxiang Zhang et al., "Toll-Like Receptor -1, -2, and -6 Polymorphisms and Pulmonary Tuberculosis Susceptibility: A Systematic Review and Meta-Analysis," *PLoS ONE* 8, no. 5 (May 14, 2013), doi:10.1371/journal.pone.0063357.
273. Christopher M. Johnson et al., "Cutting Edge: A Common Polymorphism Impairs Cell Surface Trafficking and Functional Responses of TLR1 but Protects against Leprosy," *The Journal of Immunology* 178, no. 12 (June 15, 2007): 7520–24, doi:10.4049/jimmunol.178.12.7520.
274. Mamoon Noreen and Muhammad Arshad, "Association of TLR1, TLR2, TLR4, TLR6, and TIRAP Polymorphisms with Disease Susceptibility," *Immunologic Research* 62, no. 2 (March 18, 2015): 234–52, doi:10.1007/s12026-015-8640-6.
275. Fabiana M. S. Leoratti et al., "Variants in the Toll-Like Receptor Signaling Pathway and Clinical Outcomes of Malaria," *Journal of Infectious Diseases* 198, no. 5 (September 1, 2008): 772–80, doi:10.1086/590440.
276. Lena Alexopoulou et al., "Hyporesponsiveness to Vaccination with *Borrelia burgdorferi* OspA in Humans and in TLR1- and TLR2-Deficient Mice," *Nature Medicine* 8, no. 8 (August 2002): 878–84, doi:10.1038/nm732.
277. Thomas R. Hawn et al., "A Common Human TLR1 Polymorphism Regulates the Innate Immune Response to Lipopeptides," *European Journal of Immunology* 37, no. 8 (August 2007): 2280–89, doi:10.1002/eji.200737034.
278. Katherine O. Omuetti et al., "The Polymorphism P315L of Human Toll-Like Receptor 1 Impairs Innate Immune Sensing of Microbial Cell Wall Components," *The Journal of Immunology* 178, no. 10 (May 15, 2007): 6387–94, doi:10.4049/jimmunol.178.10.6387.
279. Maximiliano Javier Jiménez-Dalmaroni, M. Eric Gerswhin, and Iannis E. Adamopoulos, "The Critical Role of Toll-like Receptors — From Microbial Recognition to Autoimmunity: A Comprehensive Review," *Autoimmunity Reviews* 15, no. 1 (January 2016): 1–8, doi:10.1016/j.autrev.2015.08.009.

280. S. Devaraj et al., "Increased Levels of Ligands of Toll-like Receptors 2 and 4 in Type 1 Diabetes," *Diabetologia* 52, no. 8 (August 2009): 1665–68, doi:10.1007/s00125-009-1394-8.
281. Karl S. Lang et al., "Toll-like Receptor Engagement Converts T-Cell Autoreactivity into Overt Autoimmune Disease," *Nature Medicine* 11, no. 2 (February 2005): 138–45, doi:10.1038/nm1176.
282. K. Stechova et al., "Healthy First-Degree Relatives of Patients with Type 1 Diabetes Exhibit Significant Differences in Basal Gene Expression Pattern of Immunocompetent Cells Compared to Controls: Expression Pattern as Predeterminant of Autoimmune Diabetes," *Scandinavian Journal of Immunology* 75, no. 2 (February 1, 2012): 210–19, doi:10.1111/j.1365-3083.2011.02637.x.
283. Hun Sik Kim et al., "Toll-like Receptor 2 Senses β -Cell Death and Contributes to the Initiation of Autoimmune Diabetes," *Immunity* 27, no. 2 (August 2007): 321–33, doi:10.1016/j.immuni.2007.06.010.
284. Li Wen et al., "The Effect of Innate Immunity on Autoimmune Diabetes and the Expression of Toll-Like Receptors on Pancreatic Islets," *The Journal of Immunology* 172, no. 5 (March 1, 2004): 3173–80, doi:10.4049/jimmunol.172.5.3173.
285. Franck J. Barrat et al., "Treatment of Lupus-Prone Mice with a Dual Inhibitor of TLR7 and TLR9 Leads to Reduction of Autoantibody Production and Amelioration of Disease Symptoms," *European Journal of Immunology* 37, no. 12 (December 2007): 3582–86, doi:10.1002/eji.200737815.
286. Swati Bhattacharyya et al., "Toll-Like Receptor 4 Signaling Augments Transforming Growth Factor- β Responses: A Novel Mechanism for Maintaining and Amplifying Fibrosis in Scleroderma," *The American Journal of Pathology* 182, no. 1 (January 2013): 192–205, doi:10.1016/j.ajpath.2012.09.007.
287. J. C. A. Broen et al., "A Rare Polymorphism in the Gene for Toll-like Receptor 2 Is Associated with Systemic Sclerosis Phenotype and Increases the Production of Inflammatory Mediators," *Arthritis & Rheumatism* 64, no. 1 (January 2012): 264–71, doi:10.1002/art.33325.

288. C. Abad et al., "Association of Toll-like Receptor 10 and Susceptibility to Crohn's Disease Independent of NOD2," *Genes and Immunity* 12, no. 8 (December 2011): 635–42, doi:10.1038/gene.2011.41.
289. Angharad R. Morgan et al., "Genetic Variation within TLR10 Is Associated with Crohn's Disease in a New Zealand Population," *Human Immunology* 73, no. 4 (April 2012): 416–20, doi:10.1016/j.humimm.2012.01.015.
290. Xiuyun Shen et al., "The Toll-Like Receptor 4 D299G and T399I Polymorphisms Are Associated with Crohn's Disease and Ulcerative Colitis: A Meta-Analysis," *Digestion* 81, no. 2 (2010): 69–77, doi:10.1159/000260417.
291. "Toll like Receptor 2 and 4 Expression in Peripheral Blood Mononuclear Cells of Multiple Sclerosis Patients. - PubMed - NCBI," accessed August 8, 2016, <http://www.ncbi.nlm.nih.gov/gate2.inist.fr/pubmed/?term=Toll+like+receptor+2+and+4+expression+in+peripheral+blood+mononuclear+cells+of+multiple+sclerosis+patients>.
292. Elena Giancchetti and Alessandra Fierabracci, "Gene/environment Interactions in the Pathogenesis of Autoimmunity: New Insights on the Role of Toll-like Receptors," *Autoimmunity Reviews* 14, no. 11 (November 2015): 971–83, doi:10.1016/j.autrev.2015.07.006.
293. Norimitsu Kadowaki et al., "Subsets of Human Dendritic Cell Precursors Express Different Toll-like Receptors and Respond to Different Microbial Antigens," *The Journal of Experimental Medicine* 194, no. 6 (September 17, 2001): 863–70, doi:10.1084/jem.194.6.863.
294. Dasgupta et al., "Plasmacytoid Dendritic Cells Mediate Anti-Inflammatory Responses to a Gut Commensal Molecule via Both Innate and Adaptive Mechanisms." *Cell Host & Microbe* 15, no. 4 (April 9, 2014): 413–23. doi:10.1016/j.chom.2014.03.006.
295. Thierry Roger et al., "Histone Deacetylase Inhibitors Impair Innate Immune Responses to Toll-like Receptor Agonists and to Infection," *Blood* 117, no. 4 (January 27, 2011): 1205–17, doi:10.1182/blood-2010-05-284711.
296. Takashi Furuta et al., "DNA Demethylation-Dependent Enhancement of Toll-like Receptor-2 Gene Expression in Cystic Fibrosis Epithelial Cells Involves SP1-Activated Transcription," *BMC Molecular Biology* 9 (2008): 39, doi:10.1186/1471-2199-9-39.

297. Nicholas Schwab et al., "An Imbalance of Two Functionally and Phenotypically Different Subsets of Plasmacytoid Dendritic Cells Characterizes the Dysfunctional Immune Regulation in Multiple Sclerosis," *The Journal of Immunology* 184, no. 9 (May 1, 2010): 5368–74, doi:10.4049/jimmunol.0903662.
298. K. Bratke et al., "Functional Expression of Granzyme B in Human Plasmacytoid Dendritic Cells: A Role in Allergic Inflammation," *Clinical & Experimental Allergy* 40, no. 7 (Luglio 2010): 1015–24, doi:10.1111/j.1365-2222.2010.03499.x.
299. Bernd Jahrsdörfer et al., "Granzyme B Produced by Human Plasmacytoid Dendritic Cells Suppresses T-Cell Expansion," *Blood* 115, no. 6 (February 11, 2010): 1156–65, doi:10.1182/blood-2009-07-235382.
300. Marie-Clotilde Rissoan et al., "Subtractive Hybridization Reveals the Expression of Immunoglobulinlike Transcript 7, Eph-B1, Granzyme B, and 3 Novel Transcripts in Human Plasmacytoid Dendritic Cells," *Blood* 100, no. 9 (November 1, 2002): 3295–3303, doi:10.1182/blood-2002-02-0638.
301. Julien J. Karrich et al., "IL-21–stimulated Human Plasmacytoid Dendritic Cells Secrete Granzyme B, Which Impairs Their Capacity to Induce T-Cell Proliferation," *Blood* 121, no. 16 (April 18, 2013): 3103–11, doi:10.1182/blood-2012-08-452995.
302. Kui Cheng et al., "Discovery of Small Molecule Inhibitors of the TLR1-TLR2 Complex," *Angewandte Chemie (International Ed. in English)* 51, no. 49 (December 3, 2012): 12246–49, doi:10.1002/anie.201204910.
303. Fabio Bagnoli et al., "Vaccine Composition Formulated with a Novel TLR7-Dependent Adjuvant Induces High and Broad Protection against *Staphylococcus Aureus*," *Proceedings of the National Academy of Sciences* 112, no. 12 (March 24, 2015): 3680–85, doi:10.1073/pnas.1424924112.
304. Theo S. Plantinga et al., "Toll-like Receptor 1 Polymorphisms Increase Susceptibility to Candidemia," *The Journal of Infectious Diseases* 205, no. 6 (March 15, 2012): 934–43, doi:10.1093/infdis/jir867.
305. Christelle Bourgeois and Karl Kuchler, "Fungal Pathogens—a Sweet and Sour Treat for Toll-like Receptors," *Frontiers in Cellular and Infection Microbiology* 2 (November 22, 2012), doi:10.3389/fcimb.2012.00142.

306. Sandra Kesh et al., "TLR1 and TLR6 Polymorphisms Are Associated with Susceptibility to Invasive Aspergillosis after Allogeneic Stem Cell Transplantation," *Annals of the New York Academy of Sciences* 1062, no. 1 (Dicembre 2005): 95–103, doi:10.1196/annals.1358.012.
307. Thierry Jouault et al., "Specific Recognition of *Candida Albicans* by Macrophages Requires Galectin-3 to Discriminate *Saccharomyces Cerevisiae* and Needs Association with TLR2 for Signaling," *The Journal of Immunology* 177, no. 7 (October 1, 2006): 4679–87, doi:10.4049/jimmunol.177.7.4679.
308. Ivana Rubino et al., "Species-Specific Recognition of *Aspergillus Fumigatus* by Toll-like Receptor 1 and Toll-like Receptor 6," *Journal of Infectious Diseases* 205, no. 6 (March 15, 2012): 944–54, doi:10.1093/infdis/jir882.
309. Katja Farhat et al., "Heterodimerization of TLR2 with TLR1 or TLR6 Expands the Ligand Spectrum but Does Not Lead to Differential Signaling," *Journal of Leukocyte Biology* 83, no. 3 (March 1, 2008): 692–701, doi:10.1189/jlb.0807586.
310. Shirong Liu et al., "TLR2 Is a Primary Receptor for Alzheimer's Amyloid β Peptide To Trigger Neuroinflammatory Activation," *The Journal of Immunology* 188, no. 3 (February 1, 2012): 1098–1107, doi:10.4049/jimmunol.1101121.
311. S. Santos-Sierra et al., "Mal Connects TLR2 to PI3Kinase Activation and Phagocyte Polarization," *The EMBO Journal* 28, no. 14 (July 22, 2009): 2018–27, doi:10.1038/emboj.2009.158.
312. Dasgupta et al., "Plasmacytoid Dendritic Cells Mediate Anti-Inflammatory Responses to a Gut Commensal Molecule via Both Innate and Adaptive Mechanisms," *Cell Host & Microbe* 15, no. 4 (April 9, 2014): 413–23. doi:10.1016/j.chom.2014.03.006.
313. Asmahan Ouabed et al., "Differential Control of T Regulatory Cell Proliferation and Suppressive Activity by Mature Plasmacytoid versus Conventional Spleen Dendritic Cells," *The Journal of Immunology* 180, no. 9 (May 1, 2008): 5862–70, doi:10.4049/jimmunol.180.9.5862.
314. Jonathan M. Pitt et al., "Fine-Tuning Cancer Immunotherapy: Optimizing the Gut Microbiome," *Cancer Research* 76, no. 16 (August 15, 2016): 4602–7, doi:10.1158/0008-5472.CAN-16-0448.

315. Jonathan M. Pitt et al., "Resistance Mechanisms to Immune-Checkpoint Blockade in Cancer: Tumor-Intrinsic and -Extrinsic Factors," *Immunity* 44, no. 6 (June 2016): 1255–69, doi:10.1016/j.immuni.2016.06.001.
316. Masanori Matsumoto et al., "Interleukin-10-Producing Plasmablasts Exert Regulatory Function in Autoimmune Inflammation," *Immunity* 41, no. 6 (December 18, 2014): 1040–51, doi:10.1016/j.immuni.2014.10.016.
317. Hao Li et al., "Interferon-Induced Mechanosensing Defects Impede Apoptotic Cell Clearance in Lupus," *Journal of Clinical Investigation* 125, no. 7 (July 1, 2015): 2877–90, doi:10.1172/JCI81059.
318. Hendrik Poeck et al., "Plasmacytoid Dendritic Cells, Antigen, and CpG-C License Human B Cells for Plasma Cell Differentiation and Immunoglobulin Production in the Absence of T-Cell Help," *Blood* 103, no. 8 (April 15, 2004): 3058–64, doi:10.1182/blood-2003-08-2972.
319. Gaetan Jego et al., "Plasmacytoid Dendritic Cells Induce Plasma Cell Differentiation through Type I Interferon and Interleukin 6," *Immunity* 19, no. 2 (August 1, 2003): 225–34, doi:10.1016/S1074-7613(03)00208-5.
320. Madhvi Menon et al., "A Regulatory Feedback between Plasmacytoid Dendritic Cells and Regulatory B Cells Is Aberrant in Systemic Lupus Erythematosus," *Immunity* 44, no. 3 (March 15, 2016): 683–97, doi:10.1016/j.immuni.2016.02.012.
321. Marijo Parcina et al., "Pathogen-Triggered Activation of Plasmacytoid Dendritic Cells Induces IL-10–Producing B Cells in Response to *Staphylococcus Aureus*," *The Journal of Immunology* 190, no. 4 (February 15, 2013): 1591–1602, doi:10.4049/jimmunol.1201222.
322. F. Machado Ribeiro and T. Goldenberg, "Mycobacteria and Autoimmunity," *Lupus* 24, no. 4–5 (April 1, 2015): 374–81, doi:10.1177/0961203314559634.
323. Henko Tadema, Peter Heeringa, and Cees GM Kallenberg, "Bacterial Infections in Wegener's Granulomatosis: Mechanisms Potentially Involved in Autoimmune Pathogenesis," *Current Opinion in Rheumatology* 23, no. 4 (July 2011): 366–71, doi:10.1097/BOR.0b013e328346c332.

324. Alexander V. Chervonsky, "Influence of Microbial Environment on Autoimmunity," *Nature Immunology* 11, no. 1 (January 2010): 28–35, doi:10.1038/ni.1801.
325. Gabriella Ruocco et al., "T Helper 9 Cells Induced by Plasmacytoid Dendritic Cells Regulate Interleukin-17 in Multiple Sclerosis," *Clinical Science* 129, no. 4 (August 1, 2015): 291–303, doi:10.1042/CS20140608.
326. Nicole Dietrich et al., "Murine Toll-Like Receptor 2 Activation Induces Type I Interferon Responses from Endolysosomal Compartments," *PLOS ONE* 5, no. 4 (April 20, 2010): e10250, doi:10.1371/journal.pone.0010250.
327. Bagnoli et al., "Vaccine Composition Formulated with a Novel TLR7-Dependent Adjuvant Induces High and Broad Protection against *Staphylococcus Aureus*." *Proceedings of the National Academy of Sciences* 112, no. 12 (March 24, 2015): 3680–85. doi:10.1073/pnas.1424924112.
328. S. Ning, J. S. Pagano, and G. N. Barber, "IRF7: Activation, Regulation, Modification and Function," *Genes and Immunity* 12, no. 6 (September 2011): 399–414, doi:10.1038/gene.2011.21.
329. Stéphane Temmerman et al., "Methylation-Dependent T Cell Immunity to *Mycobacterium Tuberculosis* Heparin-Binding Hemagglutinin," *Nature Medicine* 10, no. 9 (September 2004): 935–41, doi:10.1038/nm1090.
330. Katrina J. Falkenberg and Ricky W. Johnstone, "Histone Deacetylases and Their Inhibitors in Cancer, Neurological Diseases and Immune Disorders," *Nature Reviews Drug Discovery* 13, no. 9 (September 2014): 673–91, doi:10.1038/nrd4360.
331. Jennifer L. Brogdon et al., "Histone Deacetylase Activities Are Required for Innate Immune Cell Control of Th1 but Not Th2 Effector Cell Function," *Blood* 109, no. 3 (February 1, 2007): 1123–30, doi:10.1182/blood-2006-04-019711.
332. Hnin Thanda Aung et al., "LPS Regulates Proinflammatory Gene Expression in Macrophages by Altering Histone Deacetylase Expression," *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology* 20, no. 9 (July 2006): 1315–27, doi:10.1096/fj.05-5360com.

333. Jacqueline Neagos et al., "Epigenetic Regulation of Tolerance to Toll-Like Receptor Ligands in Alveolar Epithelial Cells," *American Journal of Respiratory Cell and Molecular Biology* 53, no. 6 (May 2015): 872–81, doi:10.1165/rcmb.2015-0057OC.

TLR2 / 1 coordonnent la réponse des cellules dendritiques plasmacytoïdes humaines aux bactéries Gram +

Les cellules dendritiques (DC) sont des cellules présentatrices d'antigène capables de façonner la réponse immunitaire adaptative en fonction des menaces qu'elles rencontrent. La nomenclature actuelle divise les DC circulantes dans le sang périphérique en trois sous-ensembles: deux sous-groupes de DC myéloïdes distingués par l'expression de BDCA-1 (CD1c) et BDCA-3 (CD141), et les pDC caractérisées par l'expression de BDCA-2 (CD303) et BDCA-4 (CD304). Les DC sont capables de capturer des antigènes et d'exprimer des molécules co-stimulatrices à leur surface; elles expriment des niveaux élevés de CMH-II, migrent vers les organes lymphoïdes secondaires et sécrètent une grande quantité de cytokines. Les pDC sont connues pour leur production très importante d'interféron (IFN) de type I et pour être en première ligne dans la détection antivirale. La dérégulation de l'activation des pDC a été observée dans différentes maladies auto-immunes (telles que le lupus érythémateux systémique, la sclérose en plaques, la sclérose systémique et le psoriasis).

Les bactéries sont généralement classées en deux groupes: GRAM+ et GRAM-. Les bactéries GRAM+ présentent différentes caractéristiques: membrane lipidique cytoplasmique, couche peptidoglycane épaisse, acides lipoteichoïques, parois cellulaires rigides et un volume de périplasme plus faible que celui des bactéries GRAM-.

Les récepteurs Toll-like (TLR) reconnaissent différents types de molécules issues d'agents pathogènes (comme les lipoprotéines, les lipides et les acides nucléiques). 10 TLR différents ont été décrits chez l'homme. Toll-Like Receptor 2 et Toll-Like Receptor 1 peuvent former des hétérodimères pour reconnaître certaines lipoprotéines bactériennes. Ces lipoprotéines sont abondantes dans les bactéries GRAM+ et sont importantes pour leur pathogénicité, leur virulence, la formation et germination de leurs spores. En outre TLR2 et TLR1 sont importants pour la reconnaissance bactérienne et les souris TLR2 knock-out ont une survie réduite.

Les différents sous-ensembles de cellules dendritiques expriment différents TLR. Les pDC expriment TLR7 et TLR9 à des niveaux élevés, mais leur expression de TLR2 ou 1 n'a pas été documentée.

La famille des gènes de l'interféron a été divisée en trois groupes distincts. Chez l'homme, la famille d'IFN de type I comprend 13 gènes d'IFN α partiellement homologues, l'IFN β et certains autres gènes qui sont mal caractérisés. L'utilisation d'IFN α/β exogène pour traiter des infections virales chez des souris a suggéré un rôle de ces cytokines dans l'immunité antivirale. Dans les infections mycobactériennes, la majeure partie des études met en évidence le rôle préjudiciable de l'IFN α/β . En 2010, le groupe O'Garra a analysé le transcriptome de grandes cohortes de patients du Royaume-Uni et d'Afrique du Sud. Ils ont identifié un profil transcriptomique induit par l'IFN α/β chez les patients présentant une tuberculose active dans le sang; cette signature était corrélée avec les paramètres cliniques. Il est intéressant de noter que ce profil réduisait à la suite d'un traitement anti-mycobactérien réussi. Cependant, si le rôle délétère de l'IFN α/β dans la tuberculose est évident, le mécanisme qui le sous-tend n'est pas entièrement élucidé. L'étude des souches hypervirulentes de *M. tuberculosis* a suggéré que l'IFN α/β jouait un rôle dans la suppression des cytokines pro-inflammatoires et de l'immunité TH1.

Pendant longtemps, les pDC étaient bien connues pour leur rôle clef dans l'immunité antivirale; cependant, peu d'études se sont penchées sur leur capacité à détecter et à répondre aux bactéries et aux champignons.

Les objectifs de ce travail de thèse sont les suivants:

1. Les pDC expriment-elles TLR2 et TLR1?
2. Les TLR2 et TLR1 sont-ils fonctionnels dans les pDC? Les pDCs répondent-elles aux lipoprotéines bactériennes?
3. TLR2 / 1 est-il impliqué dans la détection des bactéries par les pDC?
4. Les pDC sont-elles capables d'induire la polarisation des lymphocytes T?
5. Les TLR1 et TLR2 ont-ils la même fonction dans l'activation des pDC par des lipoprotéines? Ces différences se reflètent-elles sur l'activation des lymphocytes T?
6. Ces différences reposent-elles sur une signalisation intracellulaire différente?

Nous avons observé l'expression de TLR1, TLR2 (niveau basal), TLR6 et TLR10 et nous avons étudié la réponse des pDC à PAM3CSK4 (PAM3), une lipoprotéine bactérienne utilisée comme ligand prototypique de TLR1 / 2. Nous avons observé une uprégulation des molécules co-stimulatrices et de l'expression du CMH-II à la surface (FIG 1D), dans une mesure comparable aux stimuli connus de pDC, tels que le GM-CSF et le virus de la grippe. PAM3 induit une forte sécrétion d'IFN de type I et de cytokines pro-inflammatoires, bien qu'à un degré légèrement inférieur à celui du virus de la grippe (Flu) (figure 1E).

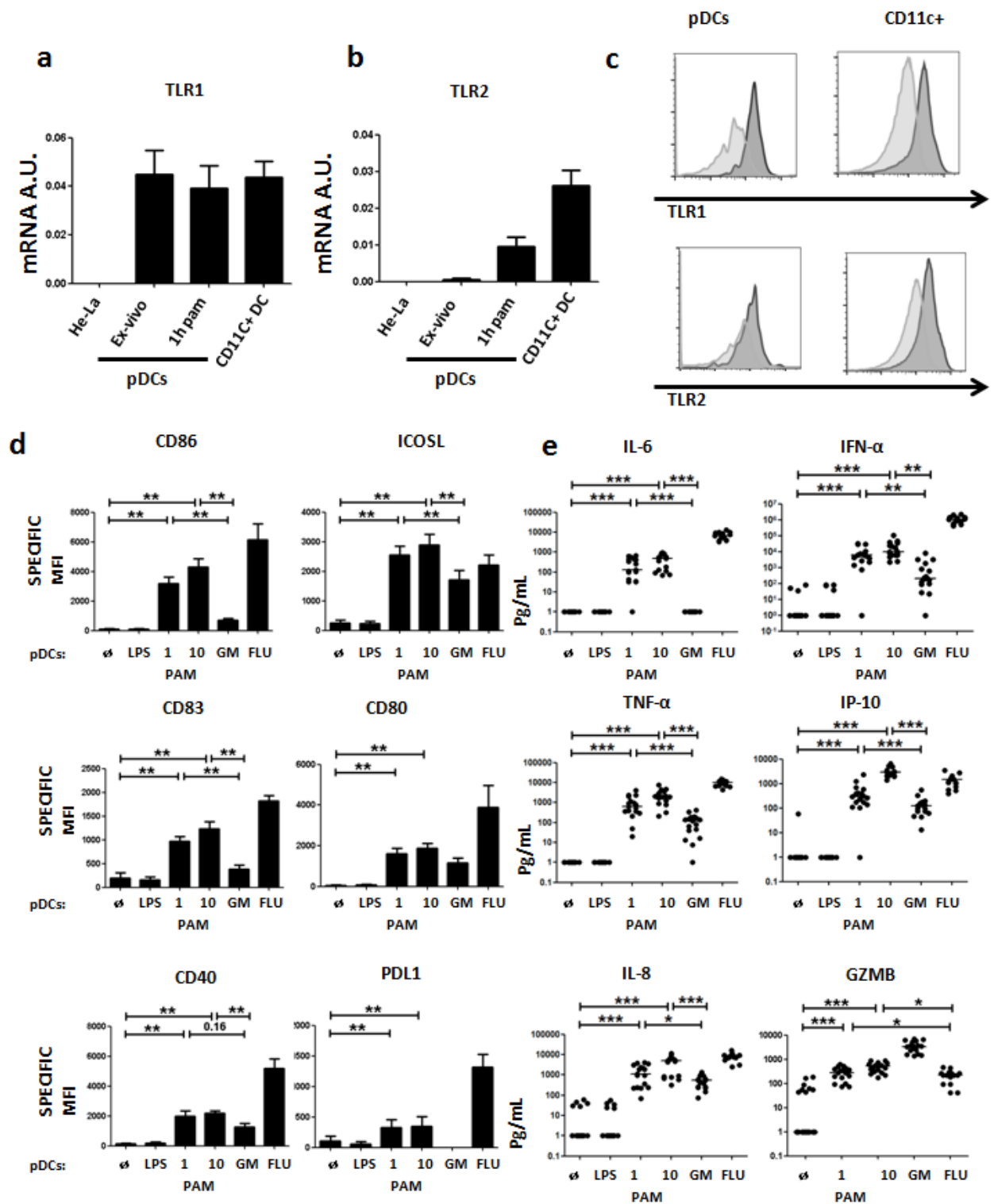


Figure 1 : Les pDC humaines expriment TLR2 / 1 et répondent à PAM3

Nous avons ensuite demandé si, en plus de la lipoprotéine purifiée, les pDC pourraient réagir de manière similaire à des bactéries GRAM+ entières (FIG. 2A, B). Les pDC ont été stimulées par trois bactéries GRAM+ tuées par la chaleur: *Mycobacterium tuberculosis*, *Staphylococcus*

aureus et *Listeria monocytogenes*. Nous avons observé une forte activation des pDC par les bactéries, avec une régulation positive des molécules co-stimulatrices et une production d'IFN de type I, à des niveaux comparables à ceux de PAM3 (figure 2A). De plus, les pDC stimulées par des bactéries GRAM+ sécrètent une quantité importante d'IFN de type I (Fig 2B). Le blocage de TLR2 / 1 réduit l'expression des molécules de costimulation induite par les bactéries GRAM+, et abroge la production d'IFN de type I (FIG2B). Les pDC activées par des bactéries GRAM+ sont capables d'induire une expansion des cellules T (figure 2C) et une prolifération (figure 2D) comparables à une stimulation virale.

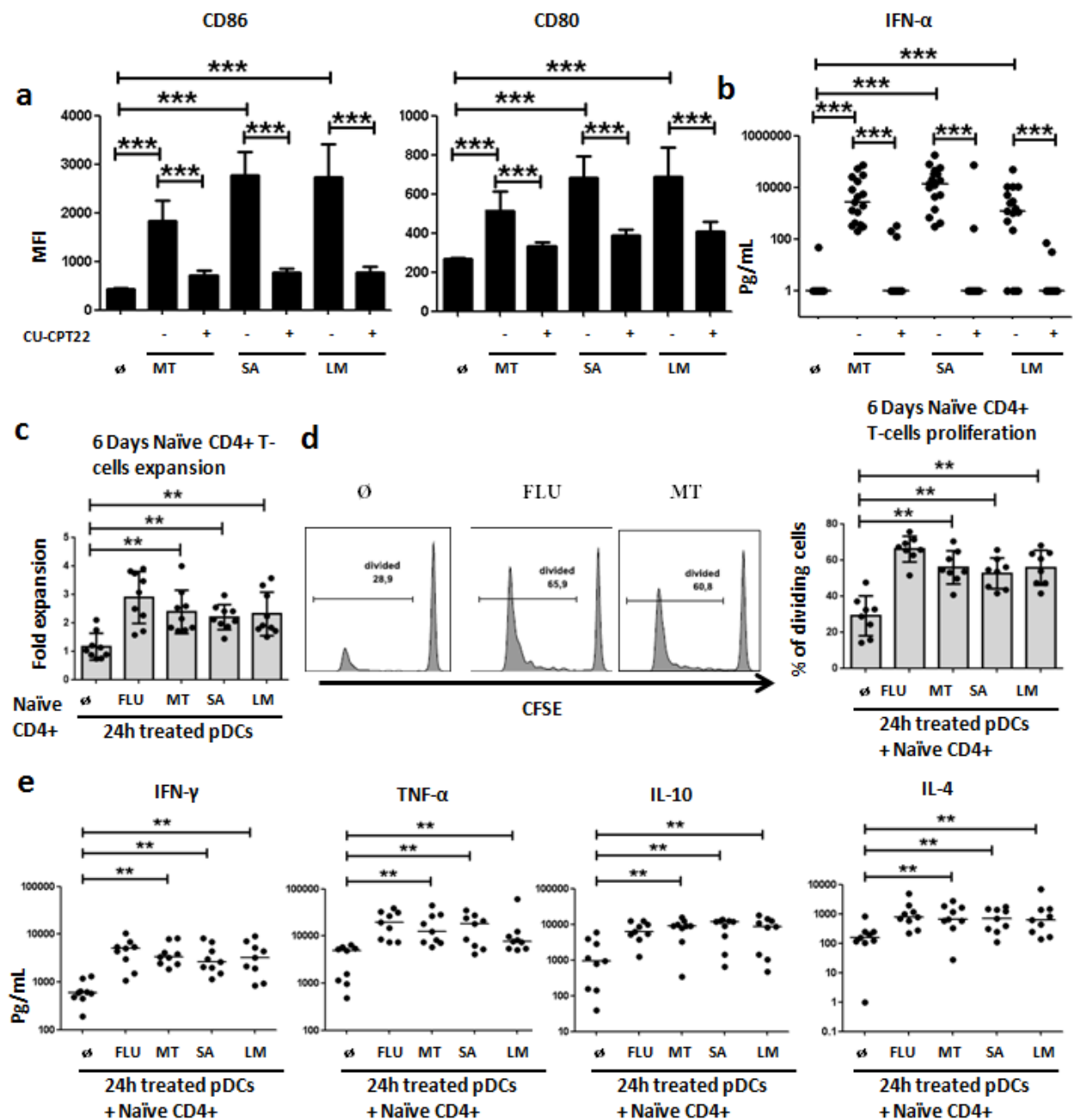


Figure 2 : Les pDC détectent différentes bactéries GRAM+ via la voie TLR2 / 1

Afin d'étudier la contribution différentielle de TLR1 et TLR2 dans la médiation de la réponse pDC à des lipoprotéines bactériennes, nous avons alternativement bloqué les deux récepteurs avec des anticorps spécifiques. Le blocage de TLR1 a affecté l'expression des molécules co-stimulatrices (CD80, CD86 et ICOSL) (FIG 3A), tandis que TLR2 n'a pas eu d'impact à ce niveau. Le blocage de TLR1 a presque supprimé la sécrétion de cytokine pro-inflammatoire (IL-6 et TNF- α) (FIG 3B). Inversement, le blocage de TLR2 a inhibé la sécrétion d'IFN de type I, qui n'était pas affectée par le blocage de TLR1 (FIG. 3B). Des expériences de co-culture entre les pDC et les lymphocytes T ont montré que le blocage de TLR1, mais pas de TLR2, inhibe l'expansion des cellules T et leur polarisation en TH2 et Treg, (FIG3C-E).

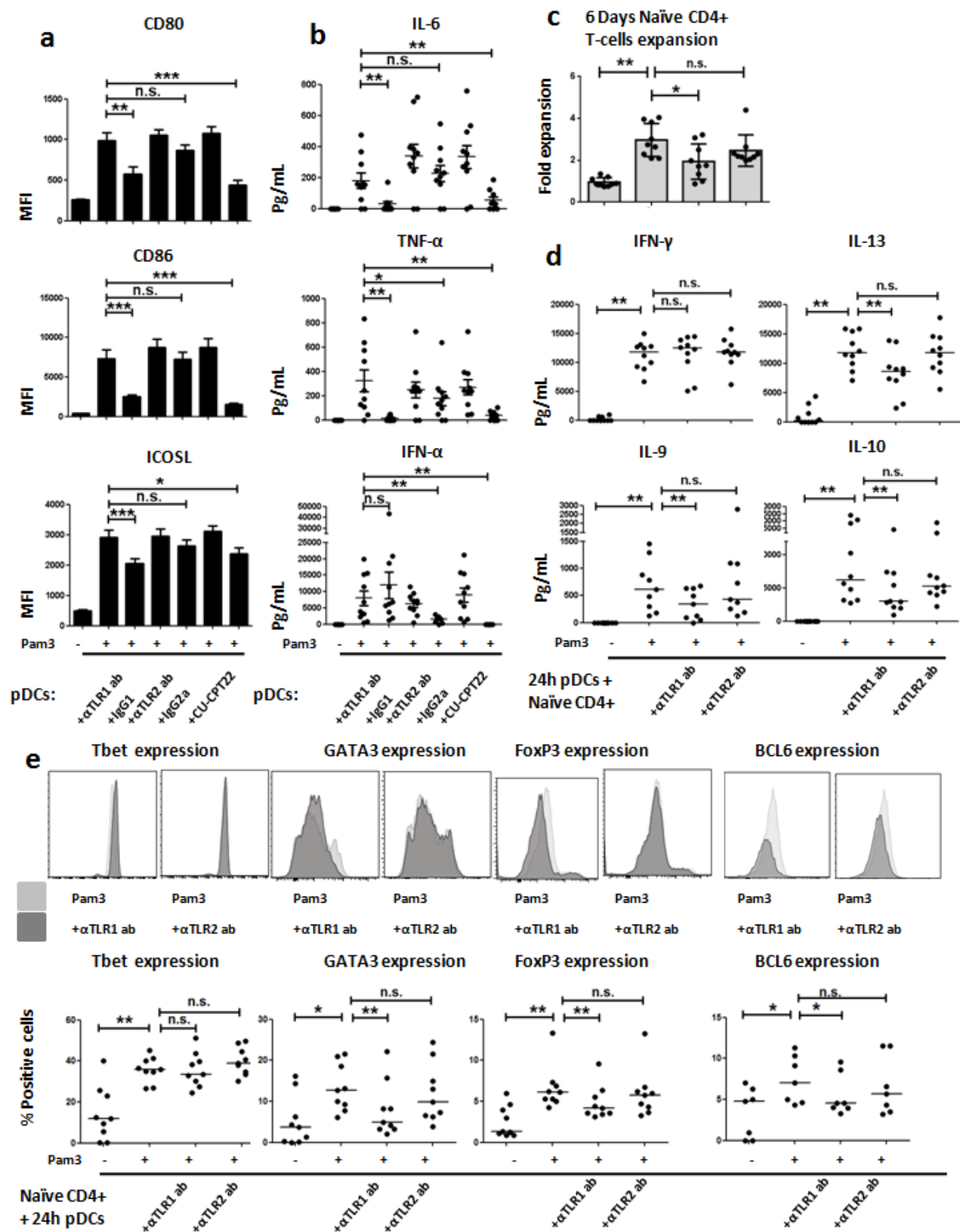


Figure 3. : Activation différentielle des pDC par TLR2 et TLR1

Nous avons également étudié le mécanisme responsable de l'activation différentielle des pDC par les voies TLR1 et TLR2. Il a été montré que l'activation de la voie MAPK et NF- κ B dans les pDC conduit à l'expression des molécules de costimulation et à la production de cytokines pro-inflammatoires, tandis que la signalisation par PI3K contrôle l'induction d'IFN de type I. Nos résultats suggèrent que les voies MAPK, NF- κ B et PI3K sont activées dans les pDC stimulées par PAM3. Le blocage des TLR1 et 2 a montré que les voies MAPK et NF- κ B sont dépendantes de TLR1, tandis que la voie PI3K est dépendante de TLR2 (FIG4A-C).

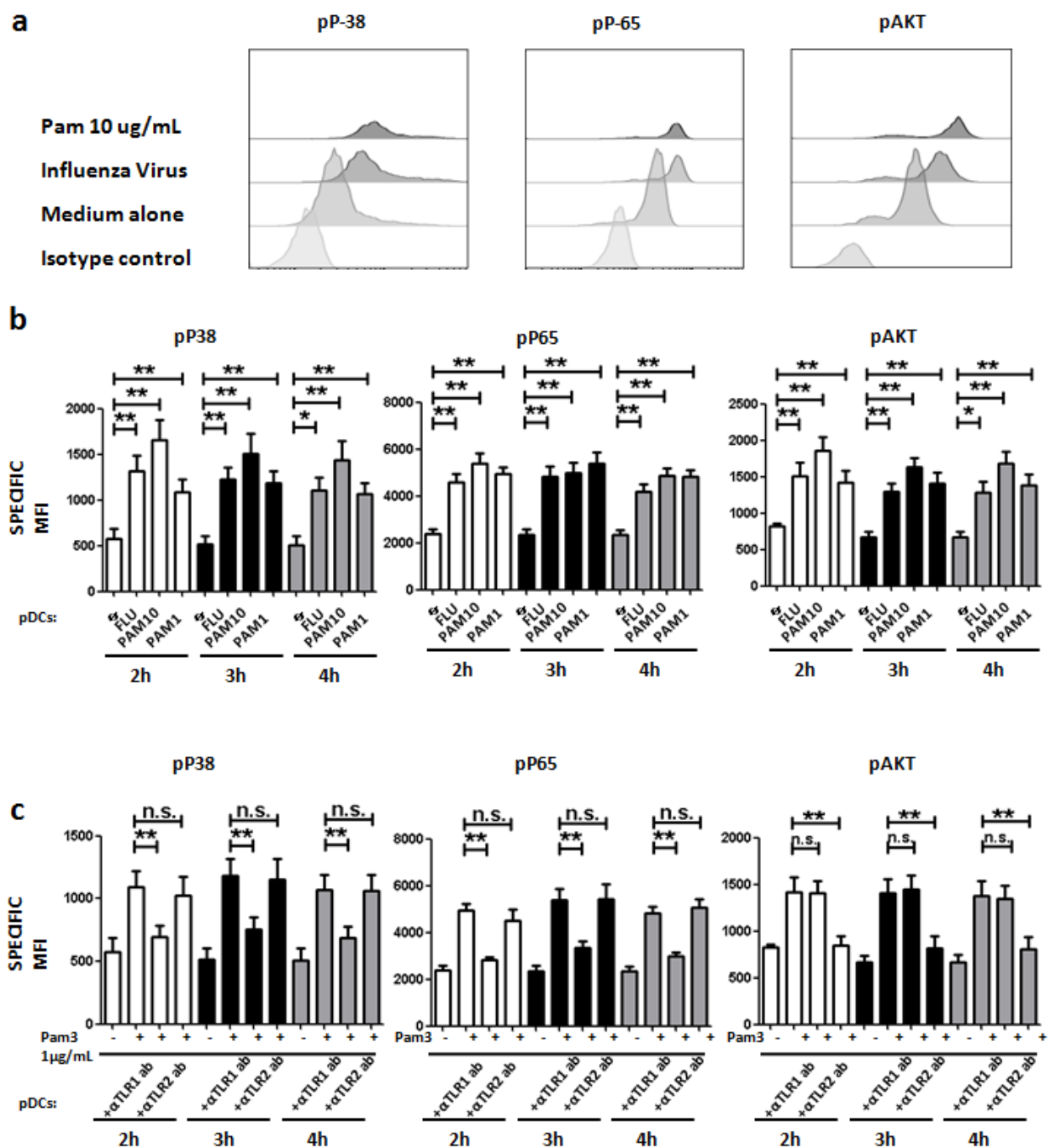


Figure 4 : Signalisation différentielle de TLR2 et TLR1 dans les pDC

En conclusion, nos résultats démontrent que les pDC expriment des TLR1 et 2 fonctionnels qui médient la réponse aux lipoprotéines bactériennes et aux bactéries GRAM+. Nous avons fourni le premier exemple de TLR de surface induisant l'interféron de type I. Cela ouvre de larges perspectives sur le rôle possible des pDC dans les maladies bactériennes. L'interaction des pDC avec les microbes commensaux semble être impliquée dans l'expansion des Treg ICOS+ au cours des traitements néo-adjuvants du cancer. Les différences observées dans l'activation médiée par TLR1 et TLR2 peuvent aider à comprendre comment ces récepteurs peuvent être impliqués dans la tolérance et l'auto-immunité, ou encore détournés par les bactéries. Nos données suggèrent que TLR1 est impliqué dans l'amorçage des lymphocytes T tandis que TLR2 joue un rôle dans l'activation de l'immunité innée par le contrôle de l'IFN de type I. En perspective, cela crée également un système gérable qui peut être exploité dans le développement de vaccins, dans l'étude du rôle de l'IFN de type I dans l'infection bactérienne, ou comme un outil pour étudier la polarisation TH.

Titre : TLR2 / 1 Orchestrent la réponse de les cellules dendritiques plasmacytoïdes humaines à les bactéries Gram +

Mots clés : humain, les cellules dendritiques plasmacytoïdes, toll like receptors, les bactéries gram-positives, détection innée

Résumé : La tuberculose, les infections par des bactéries Gram + et bactéries multi-résistantes sont des maladies mortelles dans le monde où de nouvelles études mettant en évidence le rôle pathologique interféron de type I (I IFN). Les cellules dendritiques plasmacytoïdes (pDC) produisent des quantités élevées de type I IFN suivants de détection virale. Des données récentes suggèrent que les pDC humaines pourraient détecter les bactéries. Les récepteurs de médiation de détection bactérienne dans les pDC ne sont pas connus. Au cours de ma thèse, je me suis concentré dans la caractérisation de l'expression de les récepteurs TLR2 / 1 en pDC. Ces deux récepteurs permettent pDC de détecter les lipoprotéines bactériennes.

Mon travail a montré que les pDC primaires humains expriment TLR1 et TLR2 au niveau de l'ARNm et la protéine. En les pDC primaire, je trouve que en réponse aux lipoprotéines bactériennes la régulation des molécules costimulatrices est TLR1-dépendante tandis que l'IFN-I sécrétion est TLR2-dépendante. TLR2 et TLR1 jouent un rôle différent dans les pDCs pour l'amorçage des cellules T CD4 + naïves et induisant une prolifération et la différenciation des sous-ensembles de Th1 / Th2 / Treg. Je démontre en outre que ces différences se fondent sur la voie de signalisation activés par la diversité des deux TLR. Ce travail fournit le rationnel d'explorer les pDC dans les infections bactérienne humaine.

Title : TLR2/1 orchestrate human plasmacytoid dendritic cells response to Gram+ bacteria

Keywords : humain, plasmacytoid dendritic cells, Toll Like Receptors, innate sensing, GRAM+ bacteria

Abstract : Tuberculosis, infections by Gram+ and multi-drug resistant bacteria are worldwide life-threatening diseases where new studies are highlighting the pathological role of Type I interferon (I IFN). Plasmacytoid dendritic cells (pDCs) produce high amounts of Type I IFN following viral sensing. Recent evidence suggests that human pDCs might sense bacteria. The receptors mediating bacterial sensing in pDCs are not known. During my thesis, I focused in the characterization of pDCs TLR2/1 receptors expression. These two receptors allow pDCs to sense GRAM+ bacterial lipoproteins. I show that pDCs respond to Gram+ bacteria *M. tuberculosis*, *S. aureus* and *L. monocytogenes* through TLR2/1 pathway.

My work showed that human primary pDCs express TLR1 and TLR2 at the mRNA and protein level. In human primary pDC, I found that in response to bacterial lipoproteins up-regulation of costimulatory molecules is TLR1-dependent while IFN-I secretion is TLR2-dependent. TLR2 and TLR1 play a different role in the pDCs priming of naïve CD4+ T-cells, inducing proliferation and differentiation to TH1/TH2/Treg subsets. I further demonstrate that these differences rely on the diverse signaling pathway activated by the two TLRs. This work provides the rational to explore pDCs in human bacterial infection.